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БЮЛЛЕТЕНЬ ЭКСПЕРИМЕНТАЛЬНОЙ БИОЛОГИИ И МЕДИЦИНЫ

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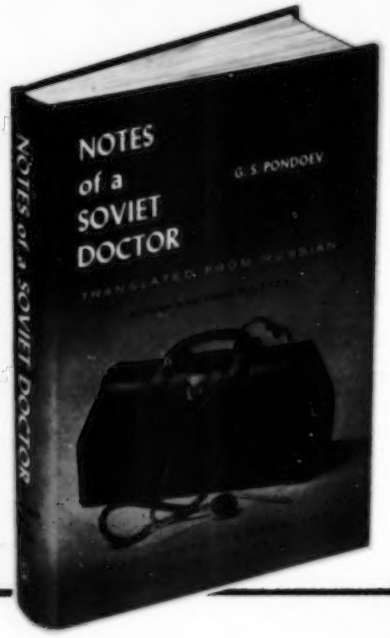

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by

G. S. PONDOEV

Honored Physician of the Georgian SSR

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the English translation
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CONTENTS

- The Vocation of the Doctor
- Not So Very Long Ago
- Medicine—Art or Science?
- Books, Lectures, Examinations
- The Doctor and the Patient
- At the Patient's Bedside
- The Doctor's Mode of Thought
- Anamnesis, Diagnosis, Prognosis
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Vol. 51, No. 6, pp. 641-746 November, 1961

CONTENTS

	PAGE	RUSS. PAGE
PHYSIOLOGY		
The Effect of Polarization on the Electrical Response of a Muscle Fiber. A. I. Shapovalov	641	3
Receptor Specificity in Various Peripheral Zones of the Interoceptive Analyzer. L. I. Ardashnikova	646	8
Sphincters of the Pulmonary Veins in Man, and Their Significance. V. S. Gurfinkel', L. L. Kapuller, and M. L. Shik	651	14
Alimentary Leucocytosis in Dogs. M. Nikš, S. Cagán, and I. Hulin	654	17
Nervous Control of the Adrenal Cortex. T. K. Dzharak'yan	661	24
PATHOLOGICAL PHYSIOLOGY AND GENERAL PATHOLOGY		
Afferent Impulses in Single Vagal Fibers in Pulmonary Damage (The Nature of Pathological Impulses). Z. N. Sergeeva, T. I. Goryunova, and S. I. Frankshtein	665	29
Changes in Tissue Oxygen Tension in Hypo- and Hyperoxia. N. V. Sanotskaya	669	33
Electrocardiogram of the Rabbit following Injury to the Myocardium and Treatment with Various Substances. N. A. Muzlaeva	673	37
The Effect of the Adrenals on Shock. V. K. Kulagin	677	42
The Effect of Adrenocorticotropic Hormone on Post-Mortem Rigidity. I. V. Markova	680	45
The Effect of Cortisone on the Course of Experimental Staphylococcal Infection. G. I. Il'in	682	46
BIOCHEMISTRY AND BIOPHYSICS		
Protein Composition of the Blood Serum in Experimental Hypertension Induced by a Partial Constriction of the Lumen of V. Porta and Ischemization of the Liver. F. A. Morokhov	685	50
The Effect of Ionizing Radiation on the Content of Free and Bound Acetylcholine in the Liver and Brain. N. N. Demin and N. V. Korneeva	689	53
Comparative Histochemical Data on Glycogen Changes in X-Ray and Strontium-90 Radiation Sickness. A. E. Ivanov and N. N. Kurshakova	692	57
The Effect of Ionizing Radiation on Electrolyte Metabolism in Rats. D. É. Grodzenskii and T. I. Ivanenko	697	62
The Influence of Radioactive Iodine upon the Nonstimulated and Stimulated Thyroid Gland. Yu. V. Krivobok	700	66
ONCOLOGY		
Metastasis Formation of Brown-Pearce Tumor Inoculated into Different Parts of the Stomach under Normal Conditions and after Exposure of the Liver to Carbon Tetrachloride. L. K. Podzei	704	70

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CONTENTS (continued)

	PAGE	RUSS. PAGE
The Growth of a Primary Tumor (Sarcoma M-1) during the Training of Rats to Hypoxia in the Low Pressure Chamber. G. N. Arkhipov	707	73
EXPERIMENTAL BIOLOGY		
Mitotic Activity during Muscular Exertion. I. A. Alov and E. N. Abramson	710	77
Changes in the Mitotic Activity of the Renal Epithelium in Relation to the Functional Activity of the Kidney. M. K. Zakharov	715	81
MORPHOLOGY AND PATHOMORPHOLOGY		
The Effect of Chondroitin Sulfate (Chonsuridum) on Collagen in Vivo. B. S. Kasavina, A. I. Rikhter, G. D. Zenkevich, and A. A. Arenberg	718	85
Multiplication of Capillaries in Different Parts of the Brain in the Postnatal Period. T. P. Zhukova	721	87
Certain Data on the Nature of Development of the Pathological Process in the Gastrointestinal Tract following Ce^{144} Administration. G. A. Lebedeva	726	93
METHODS		
Methods of Determining Specific and Nonspecific Cholinesterase in Microvolumes of Blood. A. A. Pokrovskii	730	99
The Synthesis of the Sodium Salt of β-Oxybutyric Acid from the Acetoacetic Ester. M. N. Kondrashova and I. A. Shabanova	736	104
Angiostomy Using an Isolated Segment of Small Intestine. K. V. Smirnov and V. A. Shaternikov	738	105
A Multichannel Water-Mercury Manometer. A. I. Khomazyuk	740	107

ERRATUM

Vol. 51, No. 4

The fourth paragraph on p. 503 should begin:

"After 60 days the implanted
fragments of cartilage ... "

The National Institutes of Health of the Public Health Service has supported in part the cost of publishing BULLETIN OF EXPERIMENTAL BIOLOGY AND MEDICINE through the purchase of copies for distribution to American medical libraries.

THE EFFECT OF POLARIZATION ON THE ELECTRICAL RESPONSE OF A MUSCLE FIBER

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(Presented by Active Member AMN SSSR V. V. Zakusov)

Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 51, No. 6,
pp. 3-8, June, 1961
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The dependence of the electrical response of muscle and nerve fibers on their state of polarization can give information concerning the development and spread of excitation in various synaptic structures. Coombs, Eccles and Fatt [3], using a double-barrelled, intracellular electrode, have shown that when motor neurones of the spinal cord are polarized there is a change in the transmission of excitation from one part of the nerve cell to another. Benoit and Coraboeuf [2] found a change in the amplitude of the action potentials of a muscle fiber was caused by polarization.

We have used intracellular polarization, and have led off potentials from the fiber through double-barrelled intracellular microelectrodes to record postsynaptic and spike potentials evoked by indirect stimulation.

METHOD

The experiments were carried out on isolated nerve-muscle preparations of the sartorius muscle of the grass frog. Stimulation was produced by single or repeated square-wave pulses from an electronic stimulator. The muscle potentials were led off from double-barrelled intracellular microelectrodes, as we have described in detail previously [1]. The electrodes were filled with 3 M KCl solution. The diameter of the tip was 0.5 - 1μ, and the resistance was 2-15 Mohm. One of the microelectrodes was connected to the grid of the input cathode-follower of a D. C. amplifier, and the other was connected through a 100 Mohm resistance to a source of direct current. Because of the common resistance to the two channels of the intracellular microelectrode and the capacitative connection between the channels, passing current through one channel caused a considerable fall of potential across the common resistance of the microelectrode. It was therefore difficult to determine a genuine change of membrane potential, and we studied only the magnitude of the polarizing current measured by means of an M-95 galvanometer. Currents used ranged from $1 \cdot 10^{-8}$ to $5 \cdot 10^{-7}$ a. Currents of the order of $2\text{–}5 \cdot 10^{-7}$ a were suprathreshold for some of the fibers.

RESULTS

Hyperpolarization of a muscle fiber caused by a current directed inward into the cell caused an increase in the amplitude of the action potential. The increase was from 120 to 130%, depending on the strength of the hyperpolarizing current. On the other hand, depolarization of the cell by an outwardly directed current caused a considerable fall in the spike potential. After the polarizing current had been switched off, the amplitude of the spike discharge returned to its original value.

Polarization caused changes not only in the spike but also in the electrotonic after potentials. Hyperpolarization caused an increase in the negative after potentials, while depolarization reduced them. These results confirm those of Benoit and Coraboeuf [2] on the frog muscle.

The relationship between the amplitude of the spike potentials and the degree of polarization of the cell is determined to a large extent by the ionic constitution of the surrounding medium. A calcium concentration is the most important. A reduction in the number of calcium ions had the effect that a polarizing current of a given intensity caused greater than normal change in amplitude (Fig. 1).

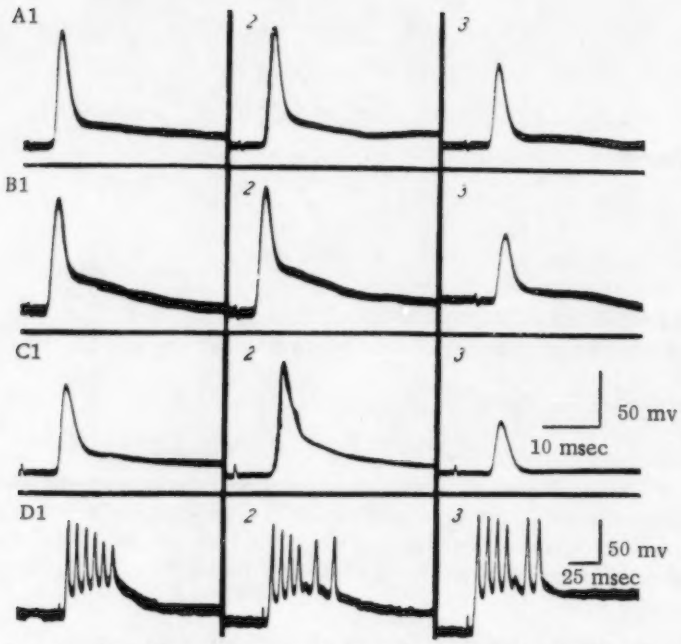

Fig. 1. The effect of polarization on spike potentials. Potentials from the nerveless part of a fiber. A) Normal calcium ion concentration: 1) normal; 2) hyperpolarizing current of $7 \cdot 10^{-8}$a; 3) depolarizing current of $8 \cdot 10^{-8}$ a; B) reduction of the calcium concentration by half: 1) normal; 2) hyperpolarizing current of $7 \cdot 10^{-8}$ a; 3) depolarizing current of $5 \cdot 10^{-8}$ a; C) calcium concentration reduced to 10%; 1) normal; 2) hyperpolarizing current of $7 \cdot 10^{-8}$ a; 3) depolarization with a current of $7 \cdot 10^{-8}$ a; D) various responses to veratrine: 1) normal; 2) hyperpolarization with current of $5 \cdot 10^{-8}$ a; 3) hyperpolarizing current of $1 \cdot 10^{-7}$ a.

The effect influenced both the increase of amplitude due to hyperpolarization and its reduction by depolarization. The marked increase in the sensitivity of the muscle fiber to polarization was noted even when the calcium ion concentration was reduced to one half. The most marked changes occured after reducing it to 10% of the normal value, or after eliminating calcium ions completely from the Ringer solution (see Fig. 1). The results of these experiments therefore agree with those obtained by Frankenhaeuser and Hodgkin [5] using the giant axon of the squid and by Frankenhaeuser [6] on the frog nerve.

Because anelectronic polarization of the cell caused a marked increase in the negative after-potential, it was pertinent to find how negative after-potentials increased by the action of drugs would be affected. We therefore studied the effect of polarization on the negative after-potential, after treatment with veratrine.

Veratrine in a concentration of $1 - 2 \cdot 10^{-6}$ caused a marked increase in the amplitude and duration of the after-potential, and as a consequence, a number of spike potentials developed at its summit. After the muscle had been treated with veratrine, the effect of a hyperpolarizing current was to cause a still greater increase in the amplitude of the after-potential, and the amplitude of the numerous discharges also increased. Similar results were obtained for the numerous responses developing as a consequence of increasing the after-potentials in a muscle fiber placed in a solution deficient in calcium ions.

Thus, increased polarization causes an increase both in transmitted and in electrotonic after-potentials.

To determine the effect of cell polarization on post-synaptic potentials, the microelectrode was placed in the region of the end-plate, as determined by the presence of "miniature end-plate potentials."

According to Fatt and Katz [4], anodic polarization of the subsynaptic region of the end-plate of a muscle fiber causes an increase in the amplitude of the end-plate potential, whereas depolarization produced the opposite effect (Fig. 2).

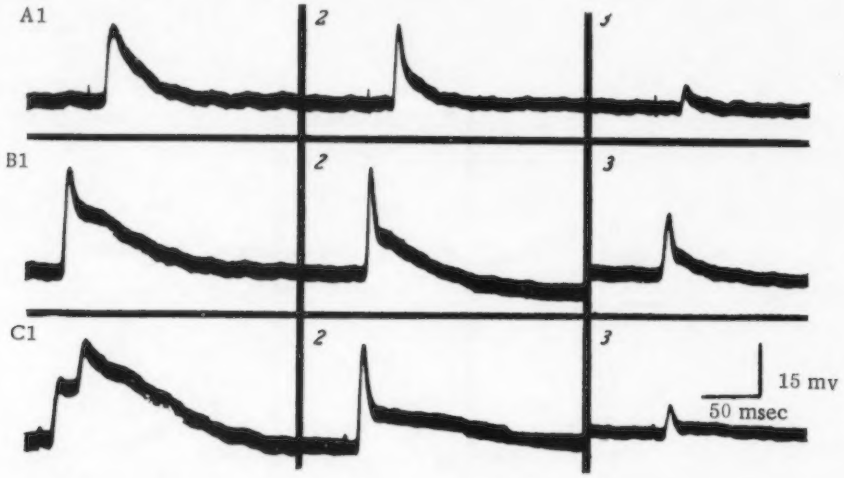

Fig. 2. The effect of polarization on end-plate potentials. A) Normal calcium ion concentration: 1) normal; 2) hyperpolarizing current of $8 \cdot 10^{-8}$ a; 3) depolarizing current of $7 \cdot 10^{-8}$; B) calcium concentration reduced to 10%: 1) normal; 2) hyperpolarizing current of $7 \cdot 10^{-8}$ a; 3) depolarizing current of $7 \cdot 10^{-8}$ a; C) after adding phenyldiguanidine ($4 \cdot 10^{-6}$ a): 1) normal; 2) hyperpolarizing current of $7 \cdot 10^{-8}$ a; 3) depolarizing current of $7 \cdot 10^{-8}$ a.

Unlike the spike potentials of a muscle fiber, during hyperpolarization there was always a shortening of the descending phase of the end-plate potential. This effect of hyperpolarization was particularly well-shown when the end-plate potential was recorded at a reduced calcium ion concentration, or when $4 - 5 \cdot 10^{-6}$ phenyldiguanidine was added to the surrounding medium. The end-plate potentials of such muscle fibers usually had a long negative plateau on the descending portion and its shape was similar to that of the negative after-potential of a spike discharge. After switching on the hyperpolarizing current, besides an increase in amplitude of the ascending portion of the end-plate potential, there was also a marked reduction of the negative plateau of the descending portion. This effect was present even when the hyperpolarization was produced by threshold currents of $1 - 3 \cdot 10^{-8}$ a. Then the reduction of the plateau took place without any change in the amplitude of the ascending portion of the end-plate potential. In some cases, after reduction of the calcium ion concentration or the addition of phenyldiguanidine, the end-plate potential not only was increased in duration, but also took on a more complex form, and additional oscillations occurred in the descending portion. Anodic polarization completely eliminated this additional negative oscillation.

On account of the marked difference in the effect of hyperpolarization on the negative after-potential occurring during the descending portion of the spike and the end-plate potential, the two processes must be different in nature. The negative after-potential which follows the spike in the denervated part of the fiber is probably due to residual permeability changes generated by the spike potential. The descending phase of the end-plate potential, particularly in cases when it is considerably lengthened and changed in shape through calcium ion insufficiency or by phenyldiguanide, is probably due to the development of a local excitatory process in the electrically excitable membrane surrounding the end-plate. Therefore, the marked reduction of the descending prolonged end-plate potential phase induced by the influence of an anode must be thought to result from a hindrance to the spread of excitation from the membrane of the end-plate to the neighboring excitable membrane.

The next step was to determine the effect of anodic polarization on the transition of the end-plate potential into a spike. Spike potentials of a muscle fiber were taken through a double-barrelled microelectrode placed near the end-plate. When the nerve-muscle preparation was in good condition, the ascending part of the spike suffered no noticeable delay. After applying anodic polarization, besides an increase in spike potential, there was also a well shown dip corresponding to a delay in the transition of the end-plate potential into a spike. Increasing the polarizing current to $1 - 4 \cdot 10^{-7}$ a caused complete disappearance of the spike potential, although the end-plate potential was still recorded (Fig. 3, A, 3).

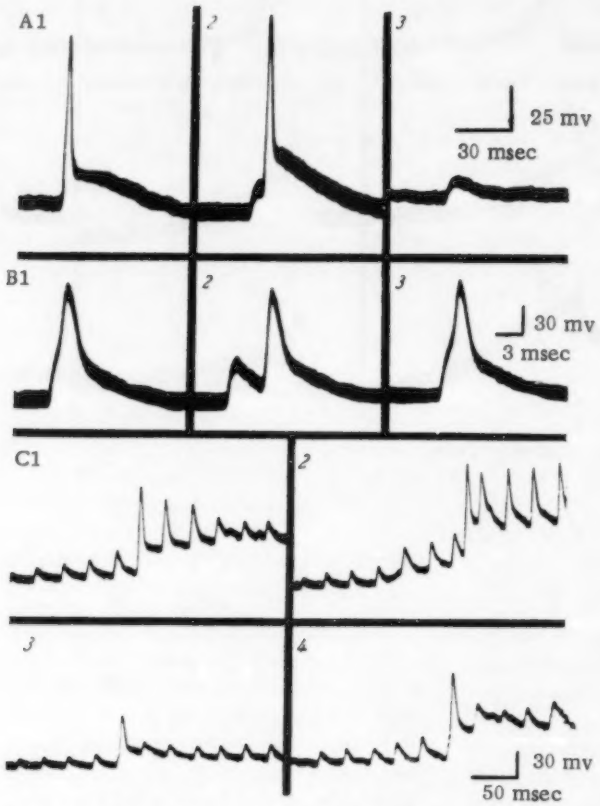

Fig. 3. The effect of polarization on single and repeated muscle fiber responses. Electrode placed near the end-plate. A: 1) normal; 2) hyperpolarizing current $8 \cdot 10^{-8}$ a; 3) hyperpolarizing current $2 \cdot 10^{-7}$ a; B: 1) normal; 2) hyperpolarizing current $6 \cdot 10^{-8}$ a; 3). normal; C: 1) normal; 2) hyperpolarizing current $8 \cdot 10^{-8}$ a; 3) depolarizing current $8 \cdot 10^{-8}$ a; 4) normal. Motor nerve stimulated at a frequency of 40 cycles.

Interference with the transmission of excitation from the end-plate on to the electrically excitable membrane was also present at strengths of hyperpolarizing current which were without effect on the amplitude of the spike potentials. A high-speed trace of this process is shown in Fig. 3, B. It can be seen that when the hyperpolarizing current is switched on, there is a scarcely noticeable dip in the ascending portion of the spike, corresponding to the transition of the local potential into a spike. With a moderate degree of hyperpolarization the time for the development of the electrical response of the electrically excitable portion of the fiber was considerably delayed. After the hyperpolarizing current had been switched off, the initial response was restored.

In connection with these results it would be interesting to determine the effect of polarization on the ability of the neuro-muscular junction to produce rhythmical stimulation. Examples of the action of hyperpolarization and depolarization on the transmission of repetitive impulses through a neuro-myal junction are shown in Fig. 3, B. It can be seen that a spike potential is not produced in response to the stimulus itself, but results from the gradual increase in the amplitude of the successive end-plate potentials (postactivational facilitation). A spike discharge occurs when the end-plate potential reaches a certain critical value. However, the amplitude of the subsequent spike discharges is rapidly reduced (pessimal effect). When there is hyperpolarization, the number of stimuli required to potentiate a spike is increased. The effect is probably due to an interference of the transmission of excitation from the end-plate on to the adjacent muscular membrane and to an increase in the critical depolarization level required for the generation of a spike. However, after this level has been reached, the spike potentials arise at each successive impulse, i. e., the pessimal effect is reduced.

On the other hand, with depolarization the critical level is reduced and the spike potential develops even more rapidly than normally, but the impulses which follow are no longer capable of generating a spike. After the polarizing current has been switched off, the initial level is restored.

Thus, with tetanic stimulation hyperpolarization may lead to a reduction, and depolarization to an increase of the pessimal effect. It must, however, be noted that the effect of polarization on the ability of the neuro-muscular junction to reproduce rhythmical impulses depends on the strength of the polarizing current. With anodic polarization of an intensity which will cause appreciable interference with the transmission of excitation from the end-plate on to the neighboring surface of the muscle fiber ($1.5 - 4 \times 10^{-7}$ a), the pessimal effect was more marked than normally.

SUMMARY

Double-barrelled intracellular microelectrodes were used to determine the effect of direct-current polarization on the electrical responses of an indirectly stimulated skeletal muscle fiber membrane. Hyperpolarization increased the amplitude of spike potentials, negative after-potentials, and end-plate potentials. Depolarization had the reverse effect. The dependence of the amplitude of the electrical responses of the muscle fiber on its polarization was most pronounced when the concentration of calcium ions in the Ringer's solution was diminished. Anodal polarization interfered with the spread of excitation from the end-plate region to the adjacent fiber membrane. Polarization influences the ability of the myoneural junction to conduct the rhythmic impulses to the motor nerve.

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RECEPTOR SPECIFICITY IN VARIOUS PERIPHERAL ZONES OF THE INTEROCEPTIVE ANALYZER

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(Presented by Academician V. N. Chernigovskii)
Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 51, No. 6, pp. 8-14, June, 1961
Original article submitted March 2, 1960

At present it is not known to what extent the different reflexogenous zones of the interoceptive analyzer are specialized to particular chemical stimuli of the internal medium.

On the one hand, various chemical and pharmacological substances such as acetylcholine, nicotine, adrenalin, serotonin, veratrine and others cause excitation of the viscera (intestine, kidneys, spleen and heart), muscles, sinocarotid and cardio-aortal regions, lesser circulation, etc. On the other hand, it may be taken as established that certain of the interoceptive zones, for example the carotid gland, are differentially sensitive to adequate stimuli and particularly to a reduced oxygen tension in the arterial blood. By recording afferent impulses in single vagal fibers it has been shown [17] that specialized receptors are present in the gastric mucosa, and that some are excited by acids and others by alkalis.

To study their differential sensitivity we have investigated the response of the intestinal chemoreceptors to two stimuli: one was hydrochloric acid, which normally reaches the intestine from the stomach, and the other was reduced oxygen tension, which is an adequate stimulus to the chemoreceptors of another zone – the sinocarotid region. As a control, experiments were also carried out in which a low oxygen tension was applied to the chemoreceptors of the carotid gland.

METHOD

The experiments were carried out on cats under urethane anesthesia. Potentials were picked up from the fine afferent nerve fibers innervating the duodenum and jejunum. In the first set of experiments the stimulus was 0.01 N or 0.1 N hydrochloric acid, and it was applied to the duodenum and jejunum while normal blood circulation was maintained; during the second set of experiments the effect of reduced oxygen concentration on the small intestine receptors was investigated. For this purpose, in some of the experiments the animals breathed a mixture of 98% nitrogen and 2% oxygen, which markedly reduced the partial pressure of oxygen in the arterial blood supply to all organs including the small intestine. In other experiments the small intestine was isolated from the general circulation and was perfused with an oxygenated Ringer-Locke solution at 37° and at constant pressure; this was periodically replaced by an oxygen-free solution.

The potentials from the afferent nerves were led off from buried electrodes similar to those used by O. M. Zamyatina [2]. They were recorded on a two-channel OB2 oscillograph constructed in the experimental works of the AMN SSSR.

RESULTS

An initial recording of the electrical activity of the afferent nerves of the duodenum and jejunum was made before applying any stimuli. In some of the experiments the amplitude of the potential oscillations was insignificant, amounting to a few micro-volts and scarcely exceeding the noise level of the amplifier (Fig. 1, a, b); in other experiments, well-marked impulses having amplitudes of the order of 10 μv were obtained (Fig. 2, a).

The injection of 1-1.5 ml of 0.01 N hydrochloric acid into the lumen of the duodenum caused an increase of impulses, or caused impulses to appear when they had previously been absent. The latent period varied from 20–30 seconds to 3–5 minutes according to the experiment, and the increase of impulses was maintained for

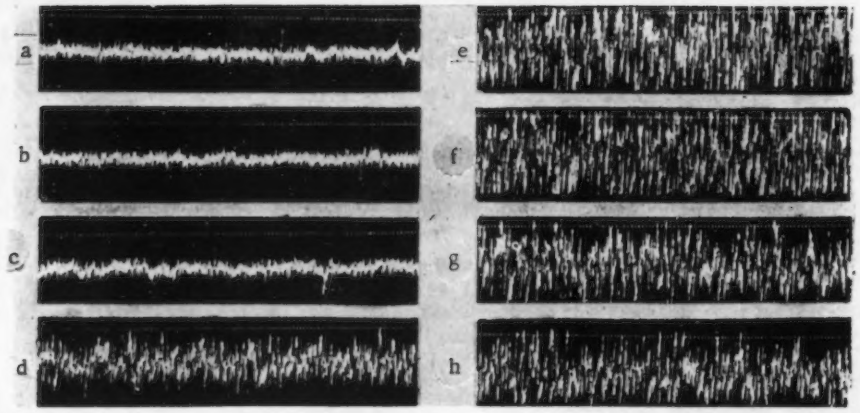

Fig. 1. Impulses in the afferent nerve from the duodenum. a) Initial level; b, c, d, e, f, g, h) 30 seconds, and 1, 2, 5, 10, 25, and 30 minutes after injecting 0.01 N HCl into the lumen of the intestine.

30 minutes or more. In Fig. 1, d, it can be seen that two minutes after applying the stimulus there is a marked increase in the amplitude of the impulses, which increases further during the next ten minutes (Fig. 1, e, f); after 10 and 20 minutes (Fig. 1, g, h) there is some falling off of the impulses, although the level remains higher than before the stimulus was applied. In the afferent nerve from the jejunum there was no noticeable change in the number of impulses.

Similar results were obtained in experiments where there was considerable electrical activity in the afferent nerves before the stimulus was applied. Again there was an increase in activity in the nerve from the duodenum, but none in that from the jejunum.

When 0.01 N hydrochloric acid was introduced into the lumen of the jejunum, there was no increase of impulses in the afferent nerve (Fig. 2, I, a – b). A marked increase could be observed only when acid of concentration 0.1 N was used. Then the latent period varied from 30 seconds to 5 minutes in different experiments. In Fig. 2, II, a – h, it can be seen that the impulses increased at the third minute, and continued to increase until the fifth and tenth minute; the rate remained high till the fifteenth and twentieth minutes, and had not returned to the original value even 60 minutes after the acid had been given.

Therefore, the action of hydrochloric acid on the mucous membrane of the duodenum and jejunum causes a marked and maintained increase in the number of impulses in the afferent nerves. However, in order to stimulate the receptors of the jejunum it was necessary to use a ten times higher concentration of the stimulus than was required for the duodenum.

It should be noted that there is a considerable interval, usually extending to several minutes, between the time at which the chemical stimulus is applied to the mucous membrane and the moment when the increase in impulses occurs. We deduced, therefore, that the excited receptors do not lie at the surface of the mucuous membrane, and the electrical activity which we recorded in the nerve fibers results from the excitation of receptors lying within the intestinal wall.

As a test we carried out several experiments in which the 0.01 N hydrochloric acid was added to the Ringer-Locke solution with which the intestine was perfused. There was then an immediate increase in the rate of impulses in the afferent nerve, and there was no definite latent period. It can be seen from Fig. 3, a, b, that there is a marked increase in the activity from the afferent nerve of the small intestine, even during the time the hydrochloric acid is being introduced into the perfusate, and the increase reaches a maximum immediately the acid ceases to be given. The increased impulsation was maintained only for 12 – 20 seconds (Fig. 3, c) and after as little as 30 seconds the nerve activity had returned to normal (Fig. 3, d).

The considerable difference in the duration of the latent period and in the period of action of the stimulus when the solution was introduced into the lumen of the intestine appears to be due to differences in the rate at which it gained access to the receptors, and in the duration of the contact. The fact that the latent period was very prolonged when the acid was introduced into the intestine, and very short when mixed with the perfusate,

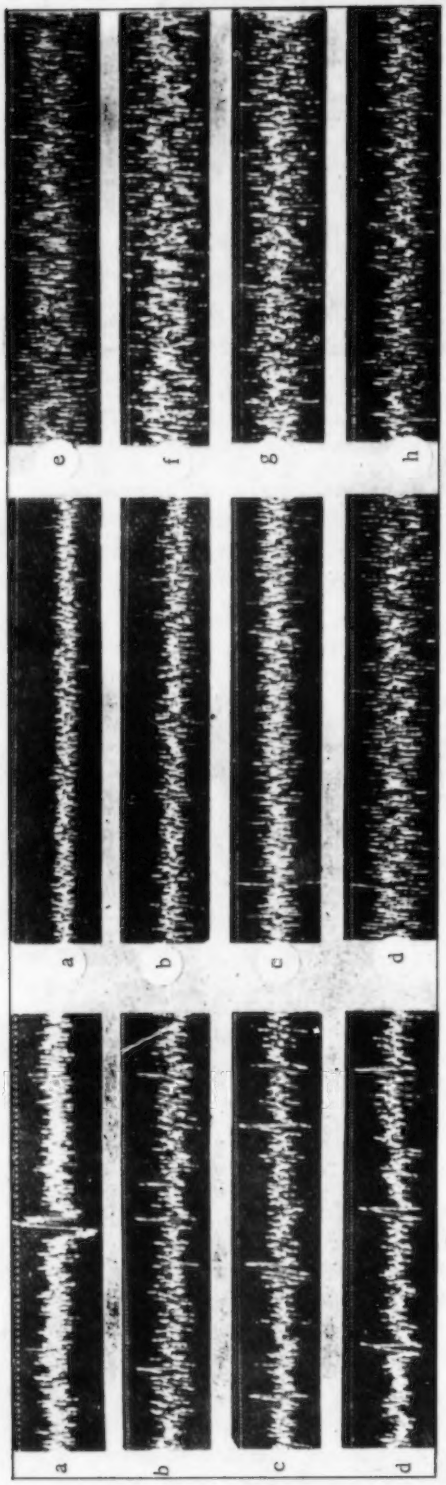

Fig. 2. Impulses in the afferent nerve from the jejunum. I a and II a) Initial level; I b,c,d,) 30 seconds, and 5 and 20 minutes, after injecting 0.01 N HCl into the intestinal lumen; II b,c,d,e,f,g,h) 2,3,5,10,15,20 and 60 minutes after injecting 0.1 N HCl into the intestinal lumen.

confirms the idea that the receptors are situated within the intestinal wall. In the second set of experiments, a simultaneous record was made of the activities in the nerves from the carotid sinus and from the jejunum when the oxygen tension in the arterial blood was reduced. After a mixture of 95-93% nitrogen with 5-7% oxygen had been breathed for only 15-20 seconds, there was an increase in the impulses from the sinus nerve indicating excitation of the chemoreceptors of the carotid gland. However, during this period and in the subsequent 3-5 minutes, during which the animal breathed the reduced oxygen mixture, there was no increase in the electrical activity in the afferent nerve from the jejunum.

A similar result was obtained in experiments in which Ringer-Locke solution containing a low partial pressure of oxygen was used to perfuse a portion of intestine which retained only nervous connection with the rest of the animal. Despite the very low oxygen partial pressure, which was obtained by raising the solution to boiling point and passing nitrogen through it for a long time, neither in the first nor in subsequent treatments was there any increase in the number of impulses. Only in two of the 40 experiments was there some slight increase in the impulses in the afferent nerve. Thus the low partial oxygen pressure which causes an increase in the electrical activity in the sinus nerve is without effect on the impulses in the intestinal nerve.

It may be concluded that the chemical receptors of the different zones have a selective sensitivity, at any rate with respect to certain adequate stimuli. A low oxygen partial pressure represents a stimulus for the receptors of the carotid gland, but does not stimulate the chemoreceptors of the intestine. In addition, even within the intestine there is also a differential sensitivity to hydrochloric acid, as between the duodenum and the jejunum.

O. N. Zamyatina [2] demonstrated excitation of the intestinal receptors occurred on feeding animals with meat and on introducing amino acids and glucose into the intestinal lumen. Her results and ours indicate that different peripheral interoceptive zones are specialized to their proper stimuli, and show little if any response to stimuli which are adequate for the chemoreceptors of a different zone. It is probable that this differential sensitivity is associated with the evolution of specialized chemoreceptive zones.

Also, a large number of studies [1, 3 – 9, 11, 15, 16, 18] have shown that many substances such as nicotine, veratrine, acetylcholine, lobeline, adrenalin, serotonin, potassium chloride, sodium bicarbonate, disodium hydrogen phosphate, and lactic, carbonic, and acetic acids stimulate all the interoceptors. Many of these stimuli are universal and excite both chemo- and mechanoreceptors disposed in different organs, including receptors of the carotid gland as well as tactile, thermal and pain receptors.

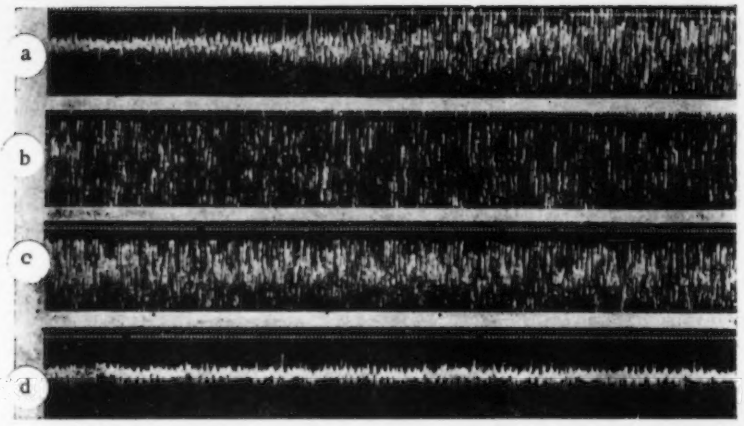

Fig. 3. Impulses in the afferent nerve from the small intestine. a) During the injection of 0.01 N HCl into the perfusate; b,c,d) 2,15 and 30 seconds after the injection.

However, many authors [10, 12, 13, 14, 19] have shown that hexamethonium, tetraethylammonium, and tubocurarine block the nonspecific sensitivity of the receptors to pharmacological stimuli, so that the latter can no longer excite all kinds of receptors; however, the sensitivity of the receptors to the adequate stimulus is preserved. For example, under the influence of hexamethonium, the receptors of the carotid gland can no longer be stimulated by acetylcholine and nicotine, but they remain sensitive to a reduced oxygen partial pressure; the baroreceptors of the carotid sinus also retain their sensitivity to pressure changes, and the tactile receptors remain sensitive to mechanical stimulation.

Thus, the exicitation which develops through the action of a number of drugs has a different mechanism from that which distinguishes the adequate stimulus. We may therefore suppose that the fact that a number of substances can stimulate all the receptors must not be interpreted as indicating that they all have a common adequate stimulus.

We have shown that the intestinal receptors are not excited by a low oxygen pressure, which is, however, an adequate stimulus for the chemoreceptors of the sinocarotid zone, and that the receptors of the duodenum and jejunum have different sensitivities to hydrochloric acid; from these and from published results we may conclude that there is a specialization of the peripheral interoceptors to different adequate stimuli.

SUMMARY

A solution of 0.01 N HCl was introduced into the lumen of the cat and it was found that there was an increase in the number of afferent impulses from the duodenum, and that a similar increase occurred when 0.1 N HCl was introduced into the jejunum. The increase in the impulses shows a long latent period lasting from 30 seconds to 5 minutes, and the effect is maintained for as long as 30 or even 60 minutes. Introduction of 0.01 N HCl solution into the perfusate provoked a brief increase of rapid onset in the afferent impulses from the jejunum. Reduction of the partial oxygen pressure of the blood or of the perfusate increased the number of impulses from the sinus nerves, but had no such effect on the nerves from the intestine. Therefore, different portions of the peripheral interoceptors show a selective sensitivity to various stimuli.

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SPHINCTERS OF THE PULMONARY VEINS IN MAN, AND THEIR SIGNIFICANCE

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(Presented by Active Member AMN SSSR V. V. Parin)
Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 51, No. 6,
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At present a great deal of intensive work is being carried out on the reflex regulation of the pulmonary circulation.

The immediate importance of such researches has increased on account of its application to the problem of pulmonary hypertension in thoracic surgery. The physiological significance of reflexes originating in the baroreceptors of the lesser circulation has been demonstrated several times [1, 3, 5]. An explanation of the significance of reflex regulation in pathology requires a knowledge of the hemodynamic conditions accompanying the pathological condition. Insufficient information is available, because until recently there was no method of studying this critically important problem.

A number of hemodynamic factors determine the condition of the vessels of the small circulation, and they include the contractile power of the right ventricle, the viscosity of the blood, the pressure in the left auricle, the functional condition of the valves of the pulmonary artery and of the sphincters of the pulmonary veins, which last factor plays a significant part. We developed this approach from studying results obtained by various authors and described by Halmagyi [4]. It has been shown that in patients with stenosis of the left atrioventricular valve, the resistance of the pulmonary vessels remains normal until the mean pulmonary capillary pressure exceeds 20 mm mercury. Not until higher pressures occur in the left auricle can changes in the pulmonary vessel occur which lead to an increase of their resistance to blood flow, which may be extremely important in some patients, and only moderate in others. Our results obtained by catheterization (23 patients), or by puncturing the vessels during operation (26 patients), completely support the results of previous investigators.

To determine whether the sphincters of the pulmonary veins play an essential part in initiating vascular changes in the lesser circulation, it was necessary to find whether they facilitate the unidirectional flow of blood from the left auricle while the pressure does not exceed 20 mm mercury, and whether they remain ineffectual at higher left auricular pressures. For this purpose it was necessary to compare curves showing the pressure in the left vein and in the left auricle in the same subjects for different values of left auricular pressure. The measurements could be made during operation, when they are necessary in order to assess the effectiveness of the mitral valvotomy.

METHOD

The needle introduced into the heart and vessels was connected to a polyethylene tube filled with physiological saline which in turn was connected to a rigid manometer. The recording system comprised an amplifier and an ink-writing oscillograph made by the firm "Eléma." The measurements were made on four patients. The parallel histological study was made of the small pulmonary arteries and veins in the lingula of the left lung, which was removed during operation.

RESULTS

Figure 1 shows pressure curves recorded in the patient B. When the pressure in the left auricle was 45/33 mm of mercury, the curve for the pulmonary vein corresponded precisely to that for the left auricle, thus indicating failure of the sphincters of the pulmonary vein. The pressure in the pulmonary artery at this time was 63/42. After mitral valvotomy, the pressure in the auricle was reduced to 37/24 mm, and the oscillations of potential in the pulmonary vein became weaker than those in the left auricle. The pressure in the left ventricle remained unchanged at 76/8 mm. The resistance of the expanded left venous aperture became less than the resistance to the

reverse flow of blood from the auricle offered by the sphincters of the pulmonary veins. The mean pressure in the left auricle had therefore to be reduced only to 30 mm in order for the sphincters to become effective again.

When there is fibrillation of the auricle, the sphincters may fail even at a mean left auricular of about 20 mm (Fig. 2, patient T).

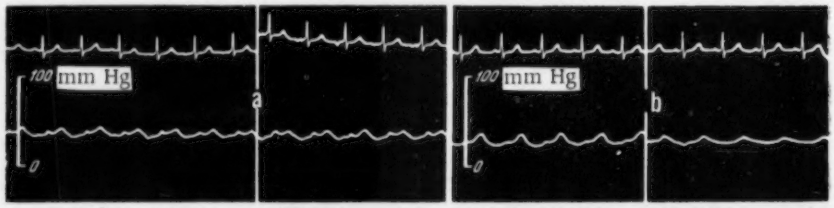

Fig. 1. Curves of the pressure in the left auricle and the pulmonary vein in the patient B during operation. a) Before mitral valvotomy; b) after the operation. Curves, from top down: electrocardiogram, pressure.

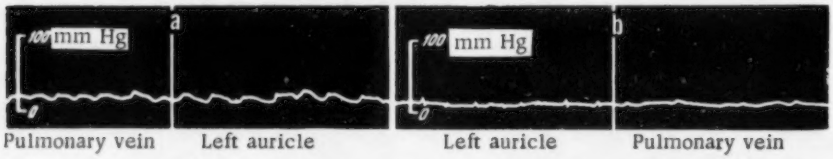

Fig. 2. Curves of the pressure in the pulmonary vein and in the left auricle in the patient T with fibrillating arrhythmia during operation. a) Before mitral valvotomy; b) after operation.

The curve of the pressure in the pulmonary vein reproduces almost precisely the pressure curve in the left auricle. This effect is combined with an early increase in the resistance of the pulmonary vessels (pressure in the pulmonary artery in this patient was 51/27 mm). The sphincters again became effective after the operation, when the pressure in the left auricle was reduced to 13/6 mm. The pressure in the left ventricle remained at its previous level of 54/2 mm.

It was shown histologically that in the small pulmonary veins there was a marked hypertrophy of the muscular layer and a considerable constriction of the lumen of the vein (Fig. 3).

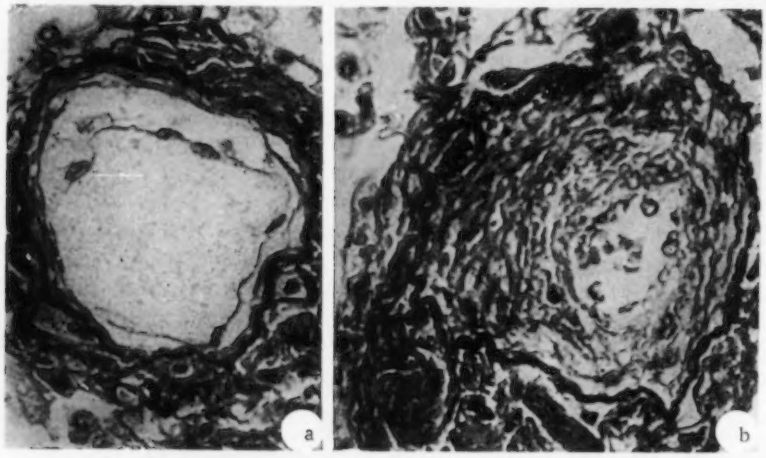

Fig. 3. Walls of the pulmonary veins: (a) a normal vein, and (b) a case of marked hypertrophy of the muscular layer and considerable constriction of the lumen. Stained in picrofuchsin and fuchselin. Magnification: objective 45 ×, ocular 6 ×.

These changes took place as a rule in cases where the mean pressure in the left auricle was above 20 mm mercury, and the sphincters of the pulmonary veins were ineffectual. In cases when the pressure in the left auricle was lower, the venous changes were weakly shown.

By preventing reverse blood flow, the sphincters of the pulmonary vein not only prevent dissipation of the work of the heart, but also protect the pulmonary capillaries from the reverse pressure wave. It would appear that failure of the sphincters, which occurs earlier in patients with auricular fibrillation, is also the factor which brings on constriction of the small intrapulmonary veins, which then take over the function of protecting the capillaries. The constriction of these veins also increases the resistance to the direct blood flow, which may lead to an increase in pressure in the pulmonary artery. The increase in pulse pressure in the latter probably leads to constriction of the pulmonary arterioles.

Auricular fibrillation, in addition to its other influences, is harmful because by putting the sphincters of the pulmonary veins out of action it brings about an early increase in the resistance of the pulmonary vessels. It is therefore very advisable to prevent auricular fibrillation during mitral valvotomy, as has been proposed by B. M. Tsukerman [2].

SUMMARY

Pressure curves in the pulmonary vein and in the left auricle were recorded in patients with stenosis of the left atrioventricular valve before and after mitral valvotomy. The curves showed that the sphincters of the pulmonary veins may fail when the mean pressure in the left auricle exceeds 20 mm Hg. Normally, by blocking the reverse flow, the sphincters protect the pulmonary vessels from the back pressure wave. With increased pressure in the left auricle, insufficiency of the pulmonary vein sphincters could provoke changes in the small pulmonary vessels, which were demonstrated histologically on biopsy material, and which had the effect of increasing the resistance to blood flow. Auricular fibrillation, by causing the sphincters to become ineffectual even when the hypertension in the left auricle is moderate, may promote a relatively early rise of pulmonary vascular resistance.

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ALIMENTARY LEUCOCYTOSIS IN DOGS

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There are numerous references [1,2,4,6,8,10,12,13,15,17,18,21,22,23,25] to control by the brain of changes in the number of leucocytes in the circulating blood induced by food as the basic stimulus. The object of our investigation has been to study complex reflex alimentary leucocytosis, in which both unconditioned and conditioned reflex activity must be involved.

METHOD

We thought it most useful to study changes in the number of leucocytes and in the differential white cell count in an experiment on sham feeding. V. G. Chizhikov [19,20] in 1921-1926 and A. R. Makarova [9] in 1956 used the method to demonstrate the reflex influence of digestion on the blood system.

In three mongrels aged 1-2 years and weighing 14-18 kg, the daily variation in the number of leucocytes was determined while the stomach was empty. During the day (between 7 a.m. and 2 p.m.) the number of leucocytes varied in different animals between plus and minus 15%. For several months they were fed once per day between 8 and 9 a.m.

An esophagotomy was then performed. Under pentothal anesthesia, an incision was made along the mid-line of the neck. A tube was then introduced into the esophagus, which was then divided between the myohyoid muscles. A longitudinal incision was now made in the esophagus and a Belous cannula of perspex introduced into it. The wound was sewn up by layers. The fistula was arranged so that apart from the experiment the animal could take fluid normally, and sham feeding could be arranged by blocking the lead-out portion of the cannula.

After sewing up the wound, and when the normal leucocyte level had been restored in all the animals, a differential white cell count was made after the animals had fasted for 24 hours.

The first blood was collected at 7 a.m. and at four subsequent 15 minute intervals; then after one hour, a further five samples were collected at half-hour intervals. Thus, in all, ten samples were collected over a period of five hours, and always from the saphenous vein.

The blood count was mainly Byurker chamber, and the cells were counted in 27 of the large squares. The differential white cell count was made in blood smears stained in Pappenheim's solution. The numbers were then given as a percentage [26].

On the following days, at 7 a.m., on each dog before feeding, sham feeding was given twice. It lasted for from 5 to 10 minutes, and the food consisted of minced meat, suet, and bread soaked in milk. During the next five hour period, the differential white count was repeated at the intervals described above.

RESULTS

In the dogs with an esophageal fistula tested while the stomach was empty, there was no change in the mean number of the leucocytes over the five hour period (the variation was between 94 and 106.7% of the original value. There was also no essential change in the differential white cell count (Table I and Fig. 1).

After sham feeding we could detect no increase in the number of leucocytes in any of the animals. When the experiment was repeated, the upper and lower limits of the count were found to be 3.8 and 8.2%, respectively (Table 2, and Fig. 1). There were no departures either in the differential white cell count from the values obtaining before feeding (see Table 2).

TABLE 1. The Number of Leucocytes and the Differential White Cell Count for Circulating Blood in Dogs Fitted with an Esophageal Fistula and Tested on an Empty Stomach (mean results of 3 studies)

Time blood was taken, min	Leucocytes per mm^3	Different cells, absolute units per mm^3 of blood					
		neutrophils		eosinophils	basophils	lymphocytes	monocytes
		lobed nuclei	rod-shaped nuclei				
0	10 483	7 862,3	765,2	41,9	0	1 048,3	765,3
15	9 917	6 386,5	694,2	29,8	29,8	1 914,0	862,7
30	11 183	7 190,7	816,4	78,3	190,1	1 711,0	1 196,5
45	9 850	6 835,9	689,5	29,5	0	1 477,5	817,7
60	10 917	7 314,4	687,8	76,4	76,4	1 965,1	796,9
90	10 317	6 943,3	897,6	72,2	30,9	1 475,3	897,7
120	10 483	6 845,4	880,6	0	31,4	1 991,8	733,8
180	10 150	6 394,5	629,2	30,5	30,5	2 233,0	832,3
240	9 850	5 782,0	591,0	98,5	29,5	2 492,1	856,9
300	11 023	6 503,6	992,1	77,2	33,0	1 907,0	1 510,1

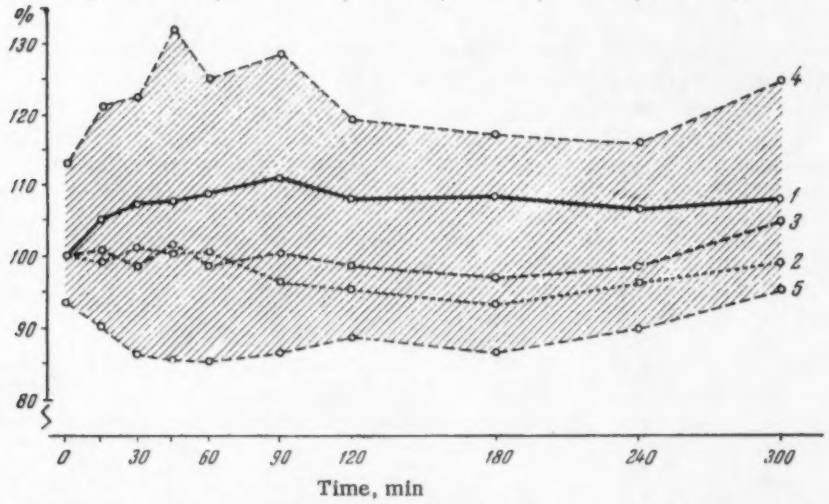

Fig. 1. Variation in the number of leucocytes in the circulating blood in dogs. 1) Mean number of leucocytes as percentage of the value before feeding (initial point of the curve) and during a 300-minute period after feeding; 2) the same before and after sham feeding; 3) changes in the number of leucocytes which were not fed during the experiment and not sham-fed; 4 and 5) mean deviations in the number of leucocytes in the above animals.

Next a further study was made on three dogs after they had been fed with 500 g of a powdered mixed food, in order to determine the extent of the leucocytic response. The maximum increase in the number of leucocytes was 12·5%, but there was no statistically significant increase (Table 3 and Fig. 1).

From Fig. 1 it can be seen that just as in the case of sham feeding, after genuine feeding there was no significant deviation of the quantities measured from those obtained when the stomach was empty. Changes in the differential white cell count do not differ essentially from those tested before feeding (see Table 3).

Statistical treatment of the results showed that there were no significant differences.

We were therefore unable to confirm the results reported in [9,20], where it was claimed that there was an alimentary leucocytosis following sham feeding in dogs.

The absence of any effect might be due to an unsuitable choice of animals. We therefore tested 12 other dogs aged from one to four years and weighing 9–23 kg. Tests were made after starving the animals for 24–48 hours, and at the same time intervals as in the previous experiments. Repeated tests were then made after feeding the

TABLE 2. Number of Leucocytes and Differential White Cell Count in the Circulating Blood of Dogs Fitted with an Esophageal Fistula Before and After Sham Feeding (mean results of 6 studies)

Time blood was taken, min	Leucocytes per mm³	Different cells, absolute units per mm³ of blood					
		neutrophils		eosinophils	basophils	lymphocytes	monocytes
		lobed nuclei	rod-shaped nuclei				
0	9 717	6 374,3	602,5	0	0	1 943,4	796,8
15	9 867	6 433,3	917,6	0	0	1 559,0	957,1
30	9 517	6 309,8	837,5	0	38,1	1 351,4	980,2
45	10 083	6 705,2	937,7	20,2	20,2	1 583,0	816,7
60	9 708	6 329,7	1 029,0	0	0	1 621,2	728,1
90	9 467	6 181,9	1 117,1	0	18,9	1 543,2	605,9
120	8 917	5 903,1	802,5	0	8,9	1 489,1	713,4
180	9 325	6 247,7	885,9	0	0	1 603,9	587,5
240	9 050	5 918,7	841,6	18,1	0	1 475,2	796,4
300	9 617	6 424,2	1 029,0	19,2	28,8	1 538,7	577,1

TABLE 3. Number of Leucocytes and Differential White Cell Count in the Circulating Blood of Dogs Fitted with an Esophageal Fistula Before and After Feeding (mean results of 7 studies)

Time blood was taken, min	Leucocytes per mm³	Different cells, absolute units per mm³ of blood					
		neutrophils		eosinophils	basophils	lymphocytes	monocytes
		lobed nuclei	rod-shaped nuclei				
0	9 993	6 365,5	689,5	30,0	10,0	2 158,5	739,5
15	10 928	6 120,0	896,0	43,7	10,9	2 939,5	917,9
30	10 571	6 554,0	909,2	52,8	0	2 071,9	983,1
45	10 528	6 874,8	852,8	31,6	0	2 063,5	705,3
60	11 093	7 332,5	809,8	11,1	11,1	1 930,2	998,3
90	10 907	6 882,3	730,8	65,4	32,7	2 290,5	905,3
120	11 243	7 071,8	798,3	33,7	0	2 282,3	1 056,9
180	10 186	6 590,3	611,2	61,1	0	2 027,0	896,4
240	10 986	6 888,2	802,0	76,9	0	2 230,2	988,7
300	10 790	6 743,7	766,1	10,8	0	2 298,3	971,1

animals with 500–1,000 g of a mixed food.

No effect was obtained in these animals, either on the number of white cells or on the differential count (Tables 4 and 5, Fig. 2).

The limits of variation in the number of leucocytes in dogs tested before they had taken food were + 2,4 and −7,7%, and in the experiments made on dogs after they had fed the limits were + 1,3 and −0,4%. From Fig. 2 it can be seen that these deviations lie within the limits for normal animals tested when the stomach was empty. In no single case was a statistically significant increase in the number of leucocytes found to occur after feeding.

According to some authors [2,3] the occurrence of an alimentary leucocytosis depends on the kind of food taken. In the last set of experiments, which were carried out on four mongrel dogs aged from one to three years and weighing 12–18 kg, we made blood tests before and after giving food consisting of protein (400 g of meat), fat (250 g of suet) and carbohydrate (250 g of glucose solution).

It can be seen from Figs. 3 and 4 that there was no statistically significant difference before and after feeding between either the numbers of leucocytes or the differential white cell counts.

There is disagreement in published reports on the time of appearance of the leucocytic reaction after feeding. Thus, A. R. Makarova [9] found an increase in the number of leucocytes to occur four hours after food had been taken.

TABLE 4. Number of Leucocytes and Differential White Cell Count in the Circulating Blood of Dogs Before and After Feeding (mean results of 22 studies)

Time blood was taken, min	Leucocytes per mm³	Different cells, absolute units per mm³ of blood					
		neutrophils		eosinophils	basophils	lymphocytes	monocytes
		lobed nuclei	rod-shaped nuclei				
0	13 886	8 692,6	791,5	69,4	27,8	3 068,8	1 235,9
15	13 943	8 700,4	822,6	125,5	41,8	3 025,7	1 227,0
30	14 060	9 082,7	815,5	253,1	42,2	2 460,5	1 406,0
45	13 829	8 988,8	885,0	152,2	41,5	2 586,0	1 175,5
60	14 043	8 931,4	940,9	126,4	28,1	2 822,6	1 193,6
90	13 857	8 937,9	859,1	180,1	13,8	2 674,4	1 191,7
120	13 891	8 876,3	833,5	125,0	27,8	2 792,1	1 236,3
180	14 064	8 818,1	857,9	84,4	14,1	2 897,2	1 392,3
240	13 961	8 809,4	949,3	83,8	27,9	2 931,8	1 158,8
300	13 848	8 765,9	872,4	138,4	27,7	2 811,1	1 232,5

TABLE 5. Number of Leucocytes and Differential White Cell Count in the Circulating Blood of Dogs Tested while the Stomach was Empty (mean results of 12 studies)

Time blood was taken, min	Leucocytes per mm³	Different cell, absolute units per mm³ of blood					
		neutrophils		eosinophils	basophils	lymphocytes	monocytes
		lobed nuclei	rod-shaped nuclei				
0	13 099	8 815,7	825,2	65,5	0,0	2 082,7	1 309,9
15	13 054	8 289,3	809,3	117,5	52,2	2 584,7	1 201,0
30	13 123	8 634,9	787,4	91,9	52,5	2 414,6	1 141,7
45	13 350	8 717,6	814,3	133,5	26,8	2 416,3	1 241,5
60	13 350	8 904,5	760,9	106,8	93,4	2 630,0	854,4
90	13 421	8 508,9	926,1	107,3	40,3	2 711,0	1 127,4
120	13 004	8 192,6	741,2	78,0	26,0	2 912,9	1 053,3
180	12 572	7 920,4	842,3	276,6	25,1	2 539,6	968,0
240	12 095	7 293,3	701,5	145,1	24,2	2 673,0	1 257,9
300	12 670	7 982,1	874,2	88,7	25,3	2 318,6	1 381,1

V. G. Chizhikov [19] found that after bread and meat had been given there were two increases in leucotyte numbers, the first occurring after 50 minutes and the second after four hours. G. S. Belen'kii [2] showed that after dogs had eaten meat or mixed food there were two phases of the leucocytic response: there was at first a leucopenia, and then a leucocytosis in which the number of leucocytes returned to the original value five hours after the food had been taken. According to S. D. Orlova [11], 30 minutes after food has been taken a leucopenia occurs, and after one hour the number of leucocytes is increased and reaches a maximun after 3-4 hours, and then returns to the original value. M. F. Sirotina [14] found that after feeding dogs with 200–250 g of meat there was a transitory leucopenia followed after two hours by a neutrophil leucocytosis. Similar results were obtained in rats after they had been given 1.5 g of bread. A. Ya. Yaroshevskii [24] fed cats with 100 g of meat and 150 ml of milk, and found that after 15 minutes there was either an increase or a decrease in the number of leucocytes. After 2-3 hours a leucocytosis occurred, which ceased after four hours.

The change occurring after 15 minutes was attributed to stimulation of the gastric interoceptors, because it was found to disappear after treating the gastric mucosa with cocaine. The leucocytosis occurring 2-3 hours after feeding was thought to result from the influence of humoral factors, because the response was maintained even after applying cocaine to the gastric mucosa.

In view of the published reports, we chose times at which blood was taken after feeding which would enable possible changes in the leucocytes to be observed. We were, however, unable to confirm the results obtained by numerous authors [17,18,23,25, etc.] on the alimentary leucocytosis in dogs. Our results agree with those of E. I.

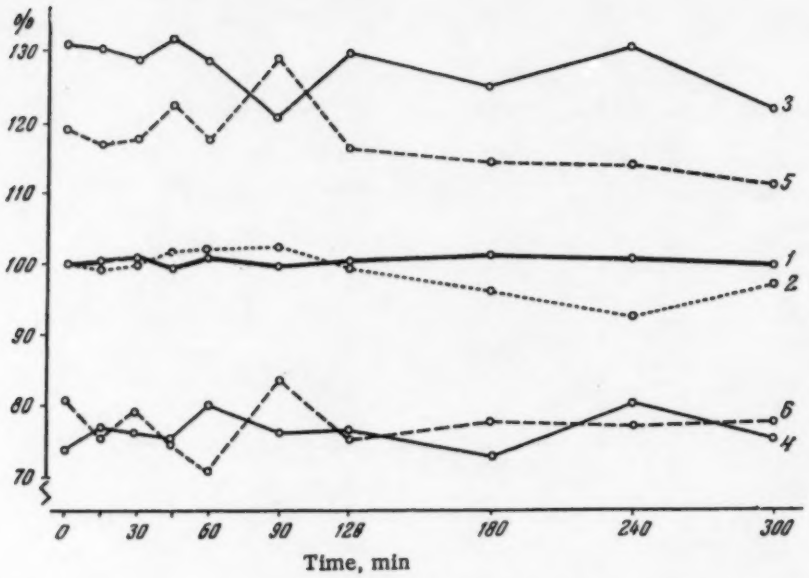

Fig. 2. Changes in the number of leucocytes in the cirulating blood in dogs. 1) Number of leucocytes (mean results for 22 experiments) as a percentage of the original value, before and after feeding; 2) the same (mean results of 12 experiments), no food taken, and no sham feeding; 3,4) mean deviations in the experiments in which food was given; 5,6) corresponding deviations in the experiments made without giving food.

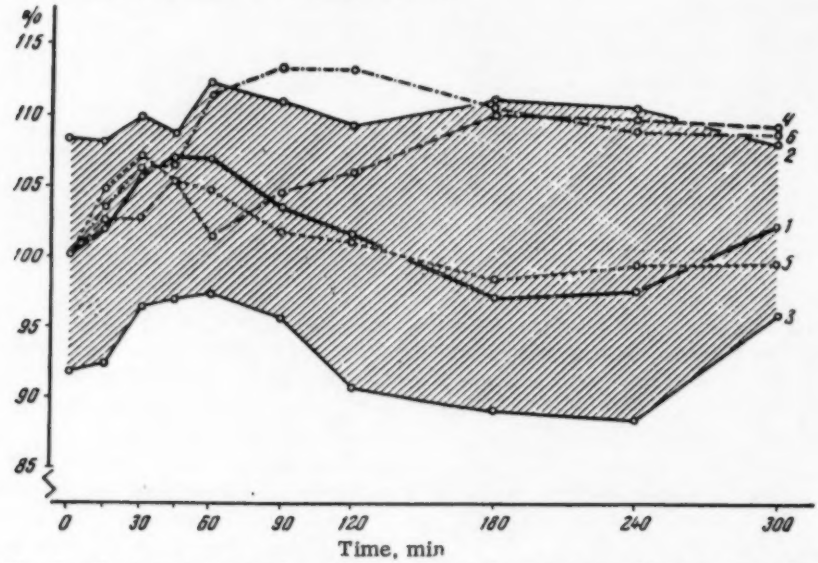

Fig. 3. Changes in the number of leucocytes in the circulating blood in dogs after receiving different kinds of food. 1) Number of leucocytes (as a percentage of the orginal value) in dogs not receiving food at the time of the experiment; 2,3) mean variations under the same conditions; 4) the same after feeding protein; 5) the same after feeding carbohydrate; 6) the same after feeding fat.

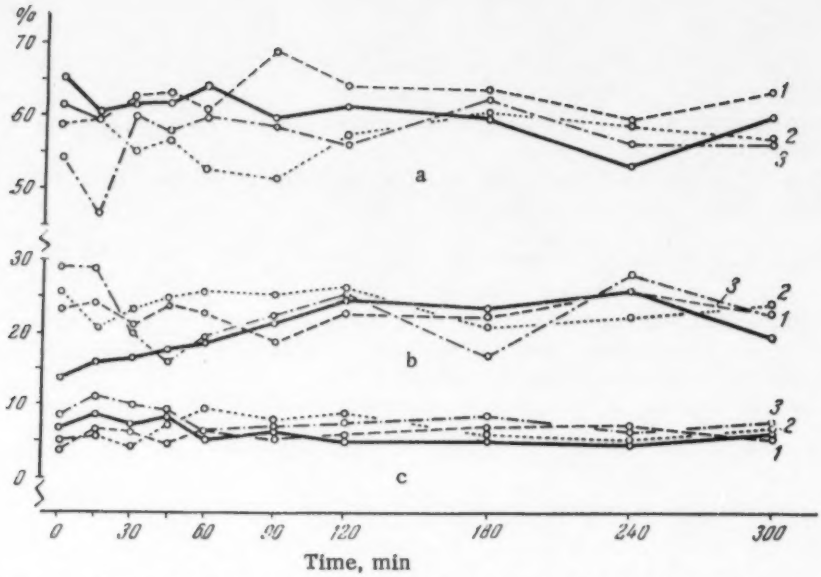

Fig. 4. Changes in the number of polymorphonuclear leukocytes (a), lymphocytes (b) and forms with rod-shaped nuclei (c) after consuming protein (1), fat (3) and carbohydrate (2). The solid line shows the number of the same kinds of cells present in the blood of dogs from whom food was withheld.

Freifel'd [16], who showed that no change in the white cells could be detected after the animals had been fed.

We consider that the difference between our results and those which had been published are due to experimental error: only small numbers of animals have been used, no statistical treatment of the results has been included and insufficient account has been taken of the variability in the number of leucocytes in the blood due to various internal and external factors.

SUMMARY

We were unable to confirm the assertions of numerous authors that an alimentary leucocytosis occurs in dogs. The reaction was studied in a sham feeding experiment.

In three dogs, after the sham feeding, there was no change in the white cell count. Repeated experiments on twelve dogs showed no significant difference between the leucocyte curves obtained before and after real feeding. The kind of food, whether protein, fat, or carbohydrate, had no influence on any change in the number of leucocytes or on the differential white cell count. The results were evaluated statistically.

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NERVOUS CONTROL OF THE ADRENAL CORTEX

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S. M. Kirov Military Medical Order of Lenin Academy, Leningrad
(Presented by Active Member AMN SSSR A. B. Lebedinskii)
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Although there is some evidence of nervous control over the adrenal cortex, no direct influence of the central nervous system has yet been demonstrated.

Recently a number of reports have been made on the pathology of the adrenal cortex, particularly in radiation sickness, and they have suggested that a parahypophyseal influence is exerted on the gland [2,6].

In acute experiments on dogs, Yu. A. Pankov [5] showed that stimulation of the splachnic nerve increased the rate of secretion of hormones from the adrenal cortex. In special experiments he demonstrated that the increase in the secretion was caused by a greater blood flow through the gland due to splanchnic stimulation.

It has been shown morphologically [3] that the adrenal cortex is rich in nerve ganglia and pathways, but shows no nerve endings. At the same time, V. I. Il'ina [3] has shown that denervation of the adrenals by section of the splanchnic nerves and removal of the semilunar ganglia causes dystrophic changes in the cortex; lipids (cholesterol) disappeared completely, and the secretory cells degenerated.

To follow up these results, we prepared to determine the effect of the nervous system on adrenal cortical function.

It is now accepted that the ascorbic acid content of the adrenals is an indication of the condition of the cortex. It is known also that immediately after giving an injection of ACTH, there is a marked reduction in the adrenal ascorbic acid content, and we have therefore studied how it is affected by denervation.

METHOD

The experiments were carried out on chinchilla rabbits weighing 2-2.5 kg.

The left adrenal was denervated in an aseptic abdominal operation performed under urethane anesthesia. The separate layers of the wound were closed by sutures, and healing took place uneventfully. After complete recovery and healing of the wound, but not until after the 15–18th day, the principal experiments were performed. The abdominal cavity was once more opened, and both adrenals removed and measurements of the ascorbic acid content made. They were weighed on a torsion balance and then triturated with sand and a 6% solution of metaphosphoric acid in twice distilled water (0.1 ml per 10 mg of tissue). The mixture was then centrifuged. One ml of the mixture was then titrated using 2,6-dichlorophenolindophenol as an indicator. An excess of indicator was added, and after 20 seconds of interaction the centrifuged fluid was extracted with isoamyl alcohol. A blank experiment was also made in which the same quantity of twice distilled water was used. The dyed extracts from the true and the blank experiment were estimated calorimetrically, and from the results the amount of ascorbic acid in the adrenals was calculated as mg percent.

RESULTS

The first control series of experiments was performed on eight healthy unoperated rabbits. These experiments were necessary to obtain information on the difference between the ascorbic acid contents of the right and left adrenals, because in the subsequent experiments the effect of the denervation was estimated in terms of the difference in vitamin C content of the innervated and denervated glands.

The results of these experiments are shown in Fig. 1.

It can be seen that the differences in ascorbic acid content between the left and right adrenals lie within the limits of ± 10%. The absolute amount of ascorbic acid varied in the different animals from 140 to 350 mg%, the

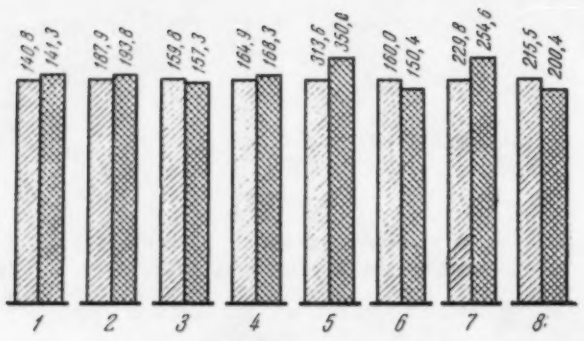

Fig. 1. Ascorbic acid content in the adrenals of the control rabbits. Columns shaded with oblique lines refer to the right adrenal (taken as 100); cross-hatched lines refer to the left adrenal (quantities expressed as a percentage of the content of the right gland); the figures above the columns indicate the amount of ascorbic acid in mg percent.

variation being probably due to individual characteristics and also to the fact that the experiments were carried out at different seasons. Other workers [1,4] have found similar values.

Section of the left splanchnic nerve did not introduce any difference in the ascorbic acid content of the left and right adrenals (Table 1).

TABLE 1. Ascorbic Acid Content of the Adrenals in Rabbits Before Partial Denervation of the Left Adrenal

Rabbit No.	Time after denervation, hr.	Ascorbic acid in the adrenal		
		right, mg%	left	
			mg %	as percentage of right adrenal content
Splanchnic nerve divided				
15	25	274,0	270,9	98
16	30	448,5	418,3	94
17	32	229,6	255,4	110
19	34	230,6	189,0	84
21	37	293,0	318,4	109
Splanchnic nerve divided, semilunar ganglion and the two upper ganglia of the abdominal sympathetic chain removed				
31	18	237,0	207,0	90
32	25	261,0	234,9	90
33	32	182,3	147,3	80
36	34	129,0	10,3	10

The observed variations did not differ from those in the control rabbits.

In two experiments prolonged electrical excitation was applied to the left splanchnic nerve; subsequent measurements revealed no difference in the adrenal ascorbic acid contents of the left and right sides.

Because the splanchnic innervation of the adrenals consists of cholinergic preganglionic fibers, in the next experiments we attempted to determine the effect on the adrenals of repeated intravenous acetylcholine injections.

The right adrenal was removed and its ascorbic acid content determined immediately, and then 5–6 injections

TABLE 2. The Effect of Repeated Acetylcholine Injections on the Adrenal Ascorbic Acid Content.

Rabbit No.	Ascorbic acid in the adrenal		
	right, mg %	left	
		mg %	as percentage of right adrenal content
18	304,0	268,0	89
20	210,0	201,0	96
22	276,7	163,0	61
23	172,0	162,2	96
24	148,8	140,3	95
25	201,6	205,2	102

of 0.4-0.6 mg acetylcholine were given at intervals of 10–15 minutes. The left adrenal was then removed, and its ascorbic acid content determined. The results of this experiment are shown in Table 2.

It can be seen from Table 2 that, as in the control experiment, the variations in the ascorbic acid content in the adrenals were within ± 10%, and only in one experiment was the left adrenal content 40% below the right.

The experiments therefore showed that as far as could be determined from the ascorbic acid content neither section of the splanchnic nerve nor its stimulation led to any change in the functional condition of the adrenal cortex.

Morphological studies [3] have shown that the adrenals are innervated by fibers entering from the semilunar ganglia. In the next set of experiments, therefore, in addition to sectioning the left splanchnic nerves, we removed the left semilunar ganglia, and also extirpated the two upper ganglia of the abdominal sympathetic chain. The experiments were performed from 18 to 36 days after the operation (see Table 1).

It can be seen from Table 1 that in all the animals the ascorbic acid content of the left adrenal fell below that of the right, though only in two animals did the difference exceed the variation found in the controls. In our opinion, the uneven results of this set of experiments indicated an incomplete denervation of some of the adrenals, due to individual variability of the course of the nerve fibers.

In order to eliminate this cause of uncertainty, in the next set of experiments, in addition to removing the sympathetic ganglia already mentioned and sectioning the splanchnic nerves, any nerve fibers which could be seen running toward the adrenals along the blood vessels were also cut. This operation was performed with the help of a binocular loupe. In the operation, carbolic acid was applied to the vessels running toward the adrenal. In carrying out manipulations near the adrenal, great care was taken to avoid any damage to it.

The adrenal ascorbic acid content in these animals was determined 20 to 65 days after the operation (Fig. 2).

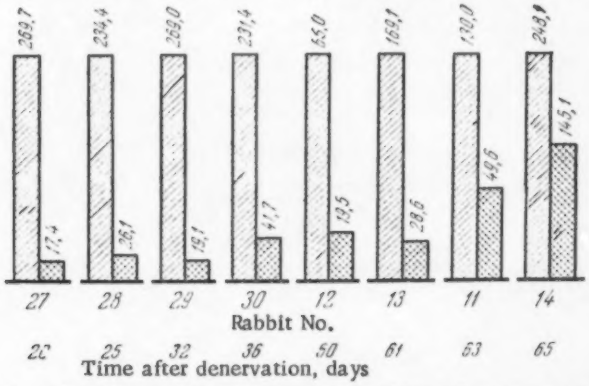

Fig. 2. Adrenal ascorbic acid content in rabbits after "complete" denervation of the left adrenal. Indications as in Fig. 1.

It can be seen from the figure that in all the animals there is a drop of 80% or more in the ascorbic acid content of the denervated gland, and the difference between the left and right glands is statistically significant (see Fig. 1). Typically, the greater differences occurred when the estimation was made early after the operation. At later times the difference was less marked and amounted only to 40–60%. Probably at these times there had been some recovery of the ascorbic acid content and some improvement in the functional condition.

Thus, the most extensive possible denervation of the adrenal gland leads to a marked change in the ascorbic acid, which must inevitably affect the production of the adrenal cortical hormone because of the important part played by vitamin C in the synthesis of corticoids.

In view of the results obtained by Yu. A. Pankova [5] quoted above, it might be thought that the changes in

the adrenal ascorbic acid content observed in our experiments were caused by changes in the blood supply to the denervated organ.

However, such a view is scarcely tenable because changes which we observed occurred between the 20th and 65th days after denervation, when the blood supply to the organ had probably been restored and when the adrenal tissues had become adapted to the new circulation.

In comparing our results with the morphological studies reported above, it seems more correct to attribute the observed changes to an arrest of central nervous trophic function.

SUMMARY

Rabbits were used to study the effect on function of denervating the adrenal gland. The amount of ascorbic acid in the gland served as an index. Twenty to sixty-five days after the splanchnic nerve had been divided, the semilunar ganglion and the two superior ganglia of the abdominal sympathetic chain excised, and the nerve fibers running to the adrenal gland along the blood vessels divided, the ascorbic acid content of the adrenal gland fell. The decrease was most marked between the 20th and 36th days. It is suggested that the CNS exerts a trophic effect on the adrenal cortex.

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AFFERENT IMPULSES IN SINGLE VAGAL FIBERS IN PULMONARY DAMAGE

(THE NATURE OF PATHOLOGICAL IMPULSES)

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(Presented by Active Member AMN SSSR V. V. Parin)

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Until recently, the reflex mechanisms in pulmonary damage could not be demonstrated without eliminating the action of the vagi [2,7,10,18,19]. However, it is not possible by this means to determine which of the pulmonary receptors are stimulated as a result of the damage, or what impulses are generated by them.

To solve this problem, a method for recording impulses in single pulmonary afferent fibers has been developed [8].

Two types of receptor have been distinguished; one responds to expansion of the lungs and the other to their contraction [8,16,17,21]. It is thought that the two receptor groups are not homogeneous, and that they vary in their degree of adaptation [13,16,21].

It is almost universally accepted that during natural breathing at the usual volume only the receptors responding to expansion are stimulated, and those which are sensitive to contraction are excited only when air is sucked out of the trachea, so that no impulses can be recorded from them during a normal expiration.

Electrical recording from single vagal fibers has been used to study respiratory changes caused by an altered composition of the inspired air [8]; by breathing ether or trichlorethylene vapor [8,20], by the action of veratrine, phenyldiguanidine and other substances [11,12,15,16,17]; in pneumothorax [1]; and in congestive pulmonary hyperemia [9,11,14].

In the present investigation, electrical recording from single vagal fibers has been used to study respiratory changes due to localized pulmonary damage.

METHOD

The experiments were carried out on cats under urethane anesthesia (1.5-2 g/kg); the thorax was intact, and respiration was maintained artificially. Local damage was produced by introducing 3 ml of water at 80–90° into the lung through the thoracic wall.

At the site of the damage there was a localized inflammatory infiltration which also contained erythrocytes; sometimes it was joined by a small atelectasis caused by an accumulation of exudate in the bronchials. Under the usual experimental conditions this damage caused an increase both in the frequency and in the depth of respiration which continued for several hours, and sometimes for 1–2 days. In our experiments, the influence of such a change on receptor function was eliminated by applying artificial respiration.

As we have shown previously [4,5] there is no appreciable change in the gaseous composition of the air in the lungs caused by localized damage, a fact which is of great importance in establishing the reflex nature of the induced respiratory changes.

The impulses were recorded from the afferent fibers of one of the vagi, most usually the right (the opposite vagus nerve was sectioned in the neck to reduce reflex influences on bronchial tone).

The splitting of the nerve was carried out in the usual way. Impulses were picked up on platinum electrodes separated by a distance of 3–5 mm. To prevent the nerve from drying up, the electrodes were covered with vaseline saturated with physiological saline. The impulses were recorded on a DIZA electromyograph. During the experiment the animals were in a screened compartment. Respiratory movements were recorded on a pneumograph.

RESULTS

By this means we were able to reveal several different reactions of the pulmonary receptors to the local damage.

Immediately after the pulmonary damage had been caused, the response to expansion of the lungs disappeared (Fig. 1).

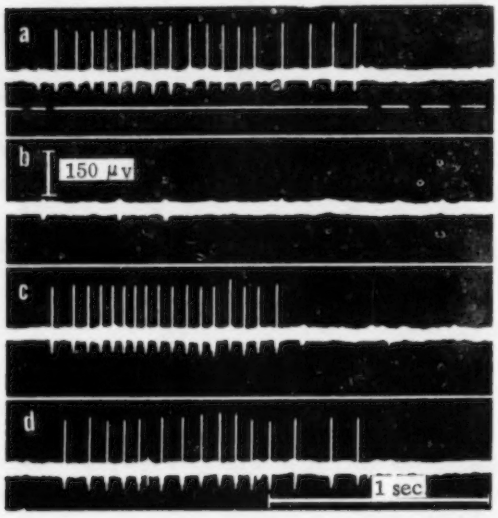

Fig. 1. Impulses from a single afferent vagal fiber recorded from the neck. A) Before pulmonary damage (impulses from stretch receptors responding at inspiration); b) after pulmonary damage (no impulses are elicited by expansion); c,d) recovery of receptor activity.

It was absent for 10–15 minutes, and then returned. Because the damaged area of the lungs was restricted, although most unlikely, there was some possibility that the impulses ceased as a result of an indirect effect on the receptor. Previously we had noticed that regional pulmonary damage, particularly in vagotomized animals,may cause a transient collapse either of the whole lung or of separate portions, and produce atelectasis beyond the damaged area. Quite possibly, therefore, the temporary stoppage of impulses from a tension receptor might result from the temporary collapse of the portion of the lung containing the receptor.

In many cases, after damage, impulses could be recorded during expiration from receptors which before damage had been excited only during inspiration (Fig. 2).

In previous investigations we have shown that beyond the damaged region, and adjacent to the atelectatic areas, there were also portions where there was a localized emphysema. Possibly impulses from a tension receptor occur at expiration by virtue of the fact that the portion of the lung in which it is situated fails to collapse, on account of a local emphysema. The maintained dilatation of these areas would then maintain the flow of impulses during expiration.

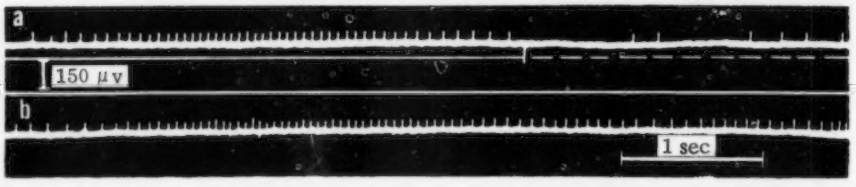

Fig. 2. Impulses from a single afferent vagal nerve fiber in the neck. A) Before pulmonary damage (impulses form a tension receptor at inspiration); b) after damage (impulses from the tension receptor are present also during inspiration).

In many experiments, after pulmonary damage, impulses of small amplitude appeared during expiration, and this effect has not previously been recorded (Fig. 3).

We have already pointed out that receptors responding to collapse are not stimulated by normal expiration, but only when air is vigorously removed from the tracheae. It is,however,known that many chemical substances such as diphenyldiguanidine, 5-hydroxytryptamine,etc., will sensitize such receptors, so that impulses may be recorded from them during a normal expiration [16]. Pulmonary damage on account of breakdown of tissue causes an increase in the amount of 5-hydroxytryptamine in the blood, and could therefore lead to sensitization of the receptors stimulated in our experiments by collapse of the lungs. On this account responses may be obtained at a volume

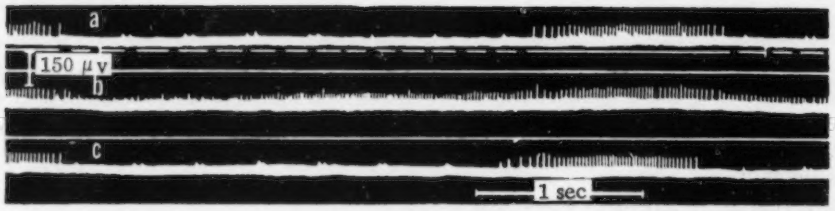

Fig. 3. Impulses from a single afferent vagal fiber in the neck. A) Before damage (impulses from a tension receptor during inspiration); b) after damage (in addition to impulses occurring during inspiration, impulses of smaller amplitude also occur during expiration); c) recovery of activity equal to that recorded before damage).

which was ineffectual before any damage had been inflicted.

There is another possible reason for impulses developing at expiration. Long ago, Adrian [8] reported that when there is infiltration of the lungs in pleuritis, receptors may respond at expiration as a result of a number of mechanical causes. This factor must also be considered in the present experiments in cases where damage caused infiltration.

Finally, in certain experiments, pulmonary damage caused a continuous low amplitude discharge, which was not associated with the respiratory cycle, to appear in fibers which were functionless before the damage. These impulses have not yet been indentified.

Our results appear to be of major importance. Until recently, the study of single-fiber activity has been made by stimulating the whole of the pulmonary receptor apparatus, by changing lung volume, by the use of inhaled drugs or chemical substances, or by congestive hyperemia. Accordingly, changes in the discharges from the receptor were always of the same kind, and consisted of either an increase or a decrease in the frequency of a respiratory volley.

In the case of localized pulmonary damage, more detailed information is available on the origin of the peripheral process transmitted to the central nervous system.

The receptors are inactive during the respiratory phase, when they would normally discharge, and begin to function when normally they would not respond.

This fact is of particular interest in explaining pathological impulses. In localized damage, particularly where the lungs are concerned, these discharges have been interpreted as arising from the damaged zone itself. Without expressing any opinion on the nature of these impulses, we must admit that the pathological condition of the periphery may be of quite a different kind, and may arise from impulses originating in receptors outside the damaged zone, whose normal functioning has been impaired by the damage.

The increase in the rate of breathing which occurs in pathological pulmonary conditions is usually attributed to an increase in the impulses from the pulmonary receptors. The results given here indicate that the pathological process disorganizes receptor activity rather than increases the rate of flow of impulses from pulmonary receptors.

SUMMARY

Recordings from single afferent vagal fibers were made after focal injury to the lungs, and revealed the following alterations in pulmonary receptor activity: 1) temporary disappearance of stretch-receptor activity, evidently due to the transient collapse of the pulmonary area containing the receptor; 2) continuation of stretch receptor activity during expiration, evidently due to the development of focal emphysema in the receptor area; 3) the development of low-amplitude activity during expiration, which was not recorded during expiration of the same volume prior to injury. The effect may very likely be explained by receptor sensitization caused by the injury. It is suggested that in addition to impulses from the pulmonary focus itself, the pathological impulses may originate from receptors beyond the focus, the effect being to disorganize rather than to intensify the discharge.

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CHANGES IN TISSUE OXYGEN TENSION IN HYPO- AND HYPEROXIA

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Results on changes in the tissue oxygen tension may be of great value in determining the oxygen supply to the tissue. Tissue oxygen tension measurements have been made possible by the method of polarography [5]. It has been used frequently in recent years to determine the effect of various factors on the oxygen tension in the brain [1,2,3,5,8] the heart [11,12] and in the skin and muscles [9,10,etc.].

It must be remembered that the changes in tissue oxygen tension may be brought about by at least three factors. There may be alteration in the blood gases, in the blood supply to the tissues, and in the rate of the oxidative processes in them. To determine the cause, the effect of each of the three factors must be determined separately.

We have studied alterations in the tissue oxygen tension caused by altering the oxygen content of arterial blood.

METHOD

Thirty-five acute experiments were carried out on cats under urethane anesthesia.

Records were made of respiration (on a pneumograph), of arterial pressure (by mercury manometer), of relative changes in the oxygen saturation of the arterial blood (using a photocell), and of the oxygen tension in skeletal muscle and in brain tissue by a polarographic method [4,6,7].

To record changes in tissue oxygen concentration we used the following electrodes: a cathode consisting of a platinum wire 0.3 mm in diameter and insulated except for the tip by a polyurethane varnish, and a nonpolarizable anode, made of a silver wire 0.5 mm in diameter, covered with a layer of silver chloride deposited electrolytically. The platinum electrode was inserted into the tissue under test, and the silver electrode into a muscle, usually at a distance of 3–4 cm from the first electrode. A potential of 0.6 v was applied to the leads (and it has been shown that when oxygen is being reduced, the current at the platinum cathode is proportional to the oxygen tension in the solution, or in the tissue [4,10]). The current and its variations were recorded by a GZS–47 galvanometer using the electrical circuit described by Montgomery and Horwitz [10].

The arterial oxygen concentration was altered by arranging for the animal to breathe mixtures containing an increased or decreased amount of oxygen from a bag.

RESULTS

It was found that in hypoxia, changes in the oxygen tension in the tissues follow the variations in the oxygen content of the arterial blood quite closely, lagging behind it somewhat in time: a reduced arterial oxygen concentration usually began 1–5 seconds after breathing a mixture poor in oxygen, and a reduction in the tension in the tissues followed a few seconds later (Fig. 1 a,b; Fig. 2 a,b).

The lag in the change of the tissue oxygen tension behind arterial tension occurred not only at the beginning but during the time that the mixture poor in oxygen is breathed: when after 1–1½ minutes the arterial tension settled at a reduced but almost constant level, the tissue tension continued to fall and settled at a fairly constant level 5–7 minutes after the reduced oxygen mixture was given.

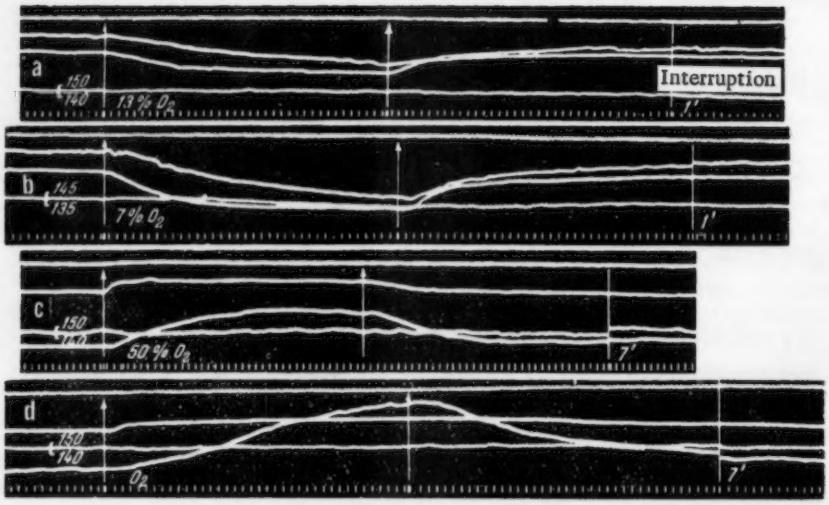

Fig. 1. Changes in oxygen concentration in the brain of a cat weighing 2.5 kg; experiment on 25/12/1959. Inspiration of mixture (↑↑), containing a) 13% of oxygen; b) 7% oxygen; c) 50% oxygen; d) pure oxygen. Curves from above downwards: a,b) respiration, oxygen tension in brain tissue; arterial oxygen saturation; arterial pressure; time marker (5 seconds); c,d) respiration, arterial blood oxygen saturation; arterial pressure; tissue oxygen tension; time marker (5 seconds).

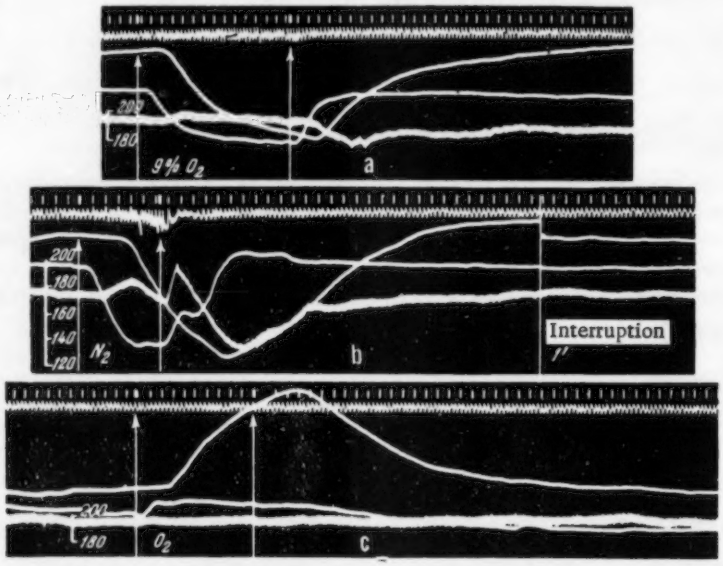

Fig. 2. Changes in oxygen tension in the gastrocnemius muscle. Experiment on cat weighing 1.9 kg, 9/10/1959. A) Breathing a mixture ( ), containing 9% oxygen; b) breathing pure nitrogen; c) breathing oxygen. Curves, from above downwards: time marker (5 seconds); respiration, oxygen tension in muscle; arterial blood oxygen concentration; arterial pressure.

The reduced arterial tension may be to some extent compensated by a reflex increase in respiration. At the same time, to prevent the reduction of the oxygen tension in the tissues a whole number of mechanisms are brought

into play, which include increase in blood supply to the tissue, and a change in the oxidative processes; however, these processes take longer to be established.

After the oxygen-deficient mixture has been replaced by normal air, the recovery of the arterial blood oxygen saturation usually occurs quite rapidly, after 30-40 seconds; only when more severe and repeated oxygen lack has been inflicted does the arterial blood oxygen saturation take longer to recover.

Even after breathing air of a reduced oxygen content for as little as 1–3 minutes, usually a longer time was required for recovery, which however was different for the different tissues. Oxygen concentration in the brain was usually restored in 3–4 minutes, and in many cases, particularly after a considerable hypoxia had developed after breathing a mixture containing 6–9% oxygen, there was an increase in the blood oxygen to a level higher than the original. The oxygen tension in muscle was frequently not fully restored in less than 6–8 minutes; only after very acute hypoxia was there sometimes a more rapid but short-lasting increase in tissue oxygen tension, after which it usually fell below the original value.

Increasing the partial pressure of oxygen in the inspired mixture (Fig. 1 c,d; Fig. 2 c) caused only small increase in the arterial blood oxygen concentration; at the same time there was a very considerable increase in the tissue oxygen tension which was better shown, the higher the concentration in the air breathed. Although when pure oxygen was given (Fig. 1 d) there was same increase in arterial blood oxygen concentration as with a 50% mixture (see Fig. 1 c), the increase in the tissue oxygen tension was better shown in the former case.

It is known that when atmospheric air is breathed, the arterial blood oxygen concentration is 95–96%; even when the partial pressure of oxygen in the inspired air is 40–50%, the hemoglobin is almost 100% saturated. When the oxygen partial pressure is increased further, its concentration in the blood plasma increases, and although no further saturation of the hemoglobin is possible, there is a considerable increase in the partial pressure of oxygen in the blood, which is particularly important in connection with its diffusion from the blood into the tissues. This circumstance explains the changes in the degree of arterial blood oxygen concentration and tissue oxygen tension as related to an increased partial pressure of oxygen in the inspired air.

When a changeover is made to breathing pure oxygen, changes take place in the same sequence as when the oxygen content of the air mixture is reduced: the changes in the tissue oxygen tension lag somewhat behind the arterial blood oxygen concentration. The same thing usually occurs at the changeover from breathing oxygen to breathing atmospheric air. However, quite frequently when the oxygen supply is removed, the fall in the tissue oxygen tension begins at the same time or even somewhat before the recovery in the saturation of the arterial blood.

The explanation is that the initial reduction in the oxygen partial pressure in the blood plasma may produce an immediate reduction in tissue oxygen tension; at the same time, the plasma concentration may be sufficiently high to produce complete saturation of the hemoglobin.

The restoration of the tissue oxygen concentration to its original level usually occurs 3–5 minutes after the cessation of breathing oxygen.

It was pointed out above that changes in tissue oxygen tension accompanying a change in the partial pressure of the air breathed lags somewhat behind changes in arterial blood oxygen concentration. Here it should be noted that this time lag is shown to a different extent in the various tissues. Thus, in the brain the tissue change occurs from 1 to 5 seconds, and usually between 2 and 3 seconds after the beginning of the change in arterial oxygen saturation (see Fig. 1), in muscle the time delay is from 6 to 20 seconds, and usually lies between 8–12 seconds (see Fig. 2).

A condition such as hypoxia produces a whole series of adaptive changes in the body, and in particular an increase in pulmonary ventilation and in the minute volume of the heart; there is also a redistribution of blood throughout the body and a change in the course of the oxidative processes.

The reason for the differences in the latent period of the oxygen tension change for the different tissues is probably that the blood supply to these tissues changes unequally, and their oxidative processes are affected differently.

The blood supply to the brain is more abudant than to the muscles, and under normal conditions is greatly increased during hypoxia and in the subsequent recovery period; the effect is due to the greater surface area of the capillaries through which diffusion of gases takes place, so that there is a greater rate of diffusion toward the surface

of the electrode. In addition, brain tissue has a higher metabolic rate, which is comparatively little reduced during hypoxia; because of the greater demand for oxygen, the drop in oxygen tension in the brain may occur earlier than in muscle.

After the oxygen deficient mixture has been withdrawn, the recovery of the oxygen tension in the brain also begins earlier (after 1–4 seconds) than it does in muscle, where it is delayed until 5–25 seconds after the onset of the recovery of the arterial blood oxygen saturation. As has already been pointed out, complete recovery of the oxygen tension in brain occurs more rapidly than in muscle. It is probable that this is mainly due to an increased blood supply to the brain, which occurs in hypoxia and in the subsequent recovery period.

SUMMARY

Experiments were performed on cats under urethane anesthesia. The effect of increased or decreased oxygen supply on oxygen tension were investigated. It was found that in hypoxia, changes in the tissue oxygen tension follow the degree of oxygen saturation of arterial blood, and are delayed slightly in time compared to the latter. The higher the oxygen concentration in the inspired air, the greater is the increase of the oxygen tension in the tissues.

The latent period for the change in oxygen tension is shorter in the brain than in skeletal muscle.

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ELECTROCARDIOGRAM OF THE RABBIT FOLLOWING INJURY TO THE MYOCARDIUM AND TREATMENT WITH VARIOUS SUBSTANCES

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It is known that after damage, heart muscle does not regenerate, and the damaged area is replaced by a scar of connective tissue [1]. The electrocardiogram then indicates the presence of a scar [6]. Our group has shown [4] that in rats the myocardium may regenerate after electrodiathermal coagulation, and that regeneration may be stimulated by means of a hydrolyzate of cardiac muscle. The electrocardiogram became normal during the time when the formation of new muscle could be observed in the center of the damage area. Then the newly formed muscle fibers were absorbed as the scar developed in a dense ridge around the damaged area. The electrocardiogram then once more showed the characteristic form associated with a scar.

The aim of the present investigation was to determine the changes in the rabbit electrocardiogram induced by the following measures: damage to the myocardium (control experiment), damage and stimulation of the regenerating myocardium, myocardial damage and prevention of the development of a scar, infliction of myocardial damage, and stimulation of regeneration to prevent the development of a scar. A hydrolyzate prepared from rabbit heart [4] was used to stimulate regeneration, and trypsin [14] was used to prevent cicatrization.

METHOD

The principal experiment was made in March and April of 1958 on 60 adult male chinchilla rabbits weighing 2·5–3·5 kg. In October, 1958, operations were made on a further 13 rabbits of the same strain and weight, and certain seasonal effects were disclosed. The control group consisted of 12 animals, and in each of these three experiments 20 rabbits were used. Before operation, the electrocardiogram was recorded without anesthesia; the electrocardiograph was an ÉKP-4m instrument, and three standard leads were used and connected with needle electrodes. The writing speed was 50 mm per second, and the sensitivity was 20 mm per mv.

Under urethane anesthesia and aseptic conditions, an incision was made precisely in the midline of the thorax, the heart was exposed, and the pericardium opened. Damage was then inflicted on the anterior wall of the left ventricle by diathermal coagulation. At the site of the damage, a white conical region developed having a depth of 5–6 mm, the apex of the cone being directed inward. The wound was sewn up in layers. Immediately after the operation, and subsequently after 1,3,5,8,12,17,24,32,45 and 64 days a record was made of the electrocardiogram. In all the experiments, the time at which the hearts were taken for fixation and the times at which the electrocardiograms were recorded were the same. In the four experiments, a total of 1149 ECG curves were taken from the three leads, and of them 766 records obtained from leads II and III were measured and studied.

After the operation, rabbits of the experimental group were treated with hydrolyzate, trypsin, and with a combination of hydrolyzate and trypsin. The hydrolyzate was prepared by our previous method [4], and 1 ml containing 40 mg of the dry substance was injected subcutaneously on the first day after the operation, and subsequently six times at intervals of 1 day. The trypsin was injected intraperitoneally on the first day after the operation, and then daily for ten days. Each dose contained 40 mg of the dry substance in 1 ml of phosphate buffer. When both substances were given, the hydrolyzate was given first, and seven days after the operation both the hydrolyzate and trypsin were given together in the same dose and at the same time as in the other two experiments.

RESULTS

The normal rabbit electrocardiogram (Fig. 1 a) has been studied in considerable detail [3, 9–12]. The frequency of the heart beat varies between 160 and 320 strokes per minute, and usually lies between 200 and 240 beats per minute. Unlike the rat electrocardiogram, the RST segment as a rule lies on the baseline, although the latter is not always well shown. The height of the R wave in the rabbit in lead II may vary between 0.07 and 0.25 mv, and usually lies between 0.1 and 0.15 mv; in lead III it varies from 0.08 to 0.35 mv, and usually lies between 0.15 and 0.20 mv.

Normally in rabbits the amplitude of the T wave is very large, particularly in lead II, and it is frequently twice as high as the QRS complex. It is rare for the T wave to be flattened so as to lie on the baseline, or to be poorly shown.

The S wave in lead II is found more frequently than it is in lead III: in 63 cases, it was well shown 19 times in lead II (30.1%), while in lead III it was found in 10 (16%) of the rabbits.

The Q wave occurs still more rarely: in lead II, out of the same 63 rabbits it occurred three times (4.8%), while in lead III it was found four times (6.3%). In these leads, the P wave was always positive, and varied within normal limits.

The published reports on the electrocardiogram in myocardial damage concern chiefly myocardial infarct induced by a ligature applied to a branch of the coronary arteries [2,3]. Some work has been done [8] on the morphological changes resulting from diathermal coagulation of the rabbit myocardium. However, no observations were made on the regeneration of muscle in the damaged zone. In [5,13], a description is given of the recovery of the electrocardiogram of animals in which the heart was damaged by resection of the ventricular wall, or by transplanting various tissues into it. In these cases recovery was quite rapid. The point where the myocardium had been cut and sutured was filled by scar tissue. Recovery of the electrocardiogram to normal also occurs in people after a penetrating myocardial wound has been sewn up [7].

Diathermal coagulation causes a marked change in the electrocardiogram which in its acute stage is very typical. The heart beat is increased on average by 9.5%. The QRS complex is greatly changed, the RST segment is displaced downward, forming an arc with a greatly deepened S wave. Similar changes take place in leads II and III. The curve has the appearance of a succession of monophasic loops. This is the acute phase (Fig. 1 b), which in rabbits lasts for a period from a few hours to 2 days after the operation.

During this time the animals were in very bad condition, they did not eat, they drank scarcely anything, and a large percentage died. In the following days, the heart beat remained high, or else very slightly slowed. The QRS complex was abnormal, in some cases the R voltage was reduced, and then, when the electrocardiogram was of an abnormal type, there was sometimes no R wave at all. The segment RST gradually returned toward the baseline; in many cases of abnormal electrocardiogram, it continued without any clear demarcation into the smooth T wave. All these changes are characteristic of the subacute phase (Fig. 1 c).

By the eighth day after the operation, the heart beat had returned to normal; subsequently there was either a small decrease or increase depending probably on the condition of the animal at the time the electrocardiogram was recorded. There then began the stage of cicatrization (Fig. 1 d), which in the electrocardiogram is shown by the return of the RST segment to the baseline; the T wave changed from the flat condition of the subacute phase to become slightly negative, and in the following days recovered until it was once more well shown. We never observed a "coronary" T wave when recording from the standard leads. The height of the R wave in the cicatricial phase is either unchanged or else reduced, though insignificantly. The cicatricial phase lasted until the end of the experiment, i.e., until the 64th day. We never observed any return, even temporary, of the electrocardiogram to normal. The electrocardiogram of another group of rabbits taken in autumn differed from that of the spring animals, which showed a smaller change of a different type; the baseline was particularly straight, and all the waves, particularly the T wave, were of smaller amplitude.

In the experiment in which the hydrolyzate was given, the electrocardiogram and the duration and sequence of the changes were similar to those in the control series. However, in the three of the nine animals which survived until this time, the electrocardiogram became normal between the 9th and 12th days, and it did not do so in the control series. There was a complete return of all the indices to their original value: the heart rate was 220–245 beats per minute, the height of the R wave in lead II was 0.1–0.18 mv, and in lead III, 0.14–0.22 mv; the ST segment was at the normal level, usually at the baseline; the T wave was no longer flattened or slightly negative as it was in

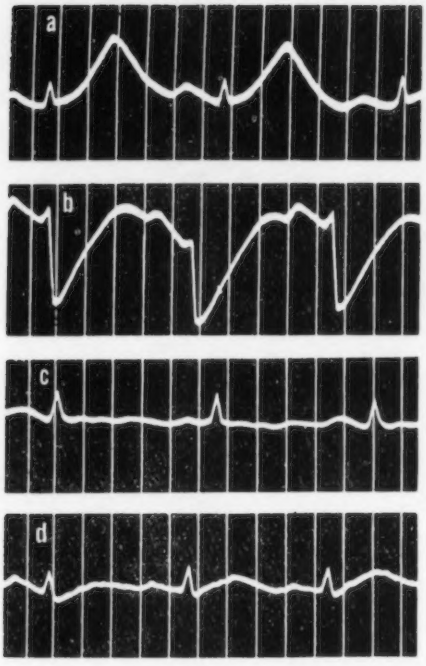

Fig. 1. Changes of the electrocardiogram of a rabbit following myocardial damage. A) Normal animal (before operation); b) immediately after operation (acute stage); c) four days after operation (subacute stage); d) 20 days after operation (cicatricial stage).

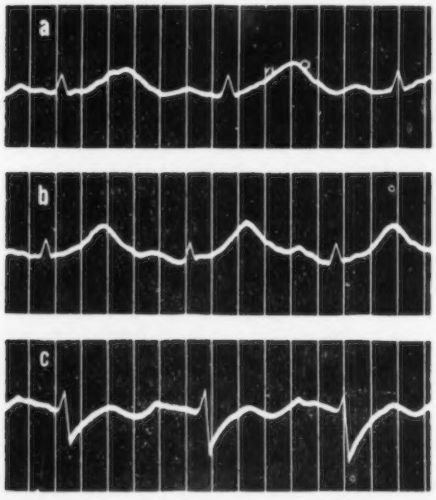

Fig. 2. Recovery of the electrocardiogram during regeneration of the myocardium. A) Normal; b) acute stage; c) 24 days after operation.

the subacute and cicatricial stages, but was well shown. However, we never observed a precise repetition of the curve exactly as it had been recorded before the operation.

It must be pointed out that, during normalization of the electrocardiogram in rats in which the myocardium had been damaged, we observed a complete repetition of the curve as recorded before damage to the heart, a result which was never obtained in rabbits. This difference in degree of recovery between rabbits and rats must be attributed to the less fixed position of the rabbit heart in the thoracic cavity, which leads to a change in the direction of the axis and so affects the electrocardiogram. In rabbits of this group, indications of a return toward normal of the electrocardiogram were maintained until the 30th-33rd day after the operation. Later, deviations from the normal occurred.

Both in these experiments on rabbits, and in our previous experiments on rats [4], where regeneration of the damaged myocardium was stimulated by hydrolyzate, the same effect occurred, and the electrocardiogram returned to normal. This effect took place in 33% of the rabbits between the 9th and 12th day after the operation. When rabbits with a damaged myocardium were treated with trypsin, again the electrocardiogram showed no pecularities which distinguished it from that of the control group, but the recovery showed constant features: it began on the 17th day after the operation and was maintained until the end of the experiment, i. e., until the 64th day after the operation, and occurred in five of the seven rabbits (71%) remaining alive at this time. The common feature of the action of trypsin on the damaged heart of both rabbits and rats is a comparatively late recovery of the electrocardiogram; in rabbits it begins on the 17th day, and in rats on the 20th. In both the electrocardiogram remains normal until the end of the experiment, i.e., in rabbits until the 64th day, and in rats until the 76th day. The percentage of rabbits in which the electrocardiogram recovered with trypsin was considerably higher than it was in rats.

In rabbits, the best result was obtained by combined treatment with hydrolyzate and trypsin. In this experiment the heart beat, the amplitude and sign of the principal waves, the position of the RST segment, and other features of the ECG, were precisely the same as they were in the controls. The duration and the sequence of the physiological stages remained normal, though the recovery period extended from the 12–18th to the 32nd-45th day after operation (Fig. 2, a, b, c).

In our previous experiments on rats with damaged hearts, when first given, the combined treatment with trypsin and vitamin B_{12} caused a 2–2 $\frac{1}{2}$-fold increase in the ECG above normal. Subsequently, the amplitude of the R wave fell to the original value, and the ECG returned to normal in all cases.

We are now in a position to compare the effects of identical substances on rabbits and rats. The hydrolyzate stimulated regeneration in rats. In 50% of the animals the ECG then became normal from the 12th day onward. In rabbits, in 33% of the cases recovery has occurred by the 9–12th day. Treatment of either rabbits or rats with trypsin caused a stable recovery lasting from the 17–20th day until the end of the experiment. However, in rats the percentage

recovering was very small, while in rabbits it was high. The combined action of treatment with a stimulant to regeneration (which in rabbits was hydrolyzate, and in our previous experiments on rats was vitamin B_{12}) led to a more marked recovery of the functional condition of the damaged myocardium than when hydrolyzate or trypsin were used separately, and in all cases the ECG returned to normal.

SUMMARY

A study was made of the ECG in normal rabbits in which diathermal injury had been inflicted on the myocardium, which was then treated with certain preparations which assisted recovery. In 33% of the cases treatment with hydrolyzate brought about recovery of the ECG between the 9–12th and 30th postoperative days. With trypsin, in 71% of the cases the ECG returned to normal between the 17th and 64th days. The combined treatment with hydrolyzate and trypsin was effective in 100% of the cases, when the ECG became normal after the 12–18th day.

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THE EFFECT OF THE ADRENALS ON SHOCK

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Recently a great deal of attention has been paid to the effect of disturbed adrenocortical function in the development of shock [1,4]. It is known that when adrenocortical function is impaired the degree of shock required to elicit trauma is reduced, and the shock is more severe. However, there is need for further information concerning the separate adrenocortical functions.

The aim of the present study has been to determine the influence of changes in the mineral and glucocorticoid adrenal components on the course of shock.

We used autografted adrenals, because from experiments which we carried out previously with D. Ya. Shurygin [3], it was found that after transplanting the adrenals, mineral corticoid function was restored within two weeks, whereas glucocorticoid function did not recover for two months. Therefore, during the first month after autografting, the two principal adrenocortical functions are dissociated.

METHOD

The experiments were carried out on 30 rats weighing 150–200 g. The adrenals were isolated, and then transplanted into the region of the superior pole of the kidneys. Shock was induced by applying special clamps for four hours to compress the soft tissue of both thighs. A study was made of the behavior of the animals, their survival time, body and rectal temperatures, the condition of the blood (red cells, leucocytes and number of eosinophils, by Dunger's method). Tests were made before applying the clamps, one hour after their application, and immediately after their removal. Three experiments were performed: 1) the control; 2) one which was carried out 12 days after autografting; and 3) a third carried out 30 days after autografting.

RESULTS

In the control group, only one rat died, 10 days after the trauma had been inflicted. Rats traumatized 12 days after the adrenals had been autografted developed severe shock and died within two days. Of the ten rats which were traumatized 30 days after autografting, three survived, and the remainder died within two days. The resistance of the autografted animals was therefore reduced, particularly in the group which were traumatized 12 days after operation. It had been shown previously that the laparotomy by itself increases resistance to trauma.

The reduced resistance must therefore be due to an impairment of adrenocortical function resulting from the autografting.

Twelve days after autografting, the wound from the operation had healed completely. The animals could be distinguished from the control group only by their behavior. They showed lassitude and reduced responses to external stimuli; these stimuli included reapplication of the clamps.

In the autografted animals, as can be seen from the table, after the trauma had been inflicted there was a marked hypothermia, particularly in the group in which the trauma was inflicted 12 days after the autografting. The difference between the control group and the normal was highly significant.

By itself, autografting caused an increase in the number of erythrocytes, evidently as a result of the blood becoming thicker. After trauma, the number in the autografted animals rose to 8,000,000 or more per mm^3.

In the control group, in many cases a leucocytosis developed after the trauma, but sometimes, on the contrary, the number of leucocytes was reduced. The change depended on the original number. Where it was originally

Changes in Body Temperature and in the Blood after Trauma in Animals with Transplanted Adrenals and in a Control Group (mean figures)

Quantity measured	Group of animals	Before traumatizing	While clamps were applied	After removing clamps
Temperature	Control	37.2± 0.70°	35.2± 1.2°	34.8± 1.3°
	Autografted, trauma after 12 days	36.6± 0.50°	29.6± 2.9°	27.7± 1.9°
	Autografted, trauma after 30 days	38.1± 0.54°	34.3± 1.1°	31.6± 1.1°
Number of erythrocytes (in millions)	Control	6.67± 0.81	6.98± 1.10	7.46± 1.17
	Autografted, trauma after 12 days	7.64± 1.30	7.75± 1.00	8.44± 1.20
	Autografted, trauma after 30 days	7.50± 0.80	8.80± 1.07	8.37± 0.87
Number of leucocytes (in thousands)	Control	18.7± 8.3	17.4± 5.4	17.2± 5.9
	Autografted, trauma after 12 days	24.5± 7.9	34.5± 9.4	25.6± 5.8
	Autografted, trauma after 30 days	18.5± 3.6	37.6± 13.6	37.3± 10.3
Number of eosinophils (median)	Control	55	44	0
	Autografted, trauma after 12 days	1240	980	460
	Autografted, trauma after 30 days	220	1890	198

relatively high, it was reduced after trauma, and vice versa. In the autografted animals, independently of what the original level was, in all the experiments there was a marked leucocytosis and the number of white cells increased to 25,000–37,000 per mm^3. The increase was best shown in the animals in which the trauma was inflicted 30 days after autografting.

In the autografted animals, the initial number of eosinophils was considerably higher than in the control group, showing that the trauma had not caused any marked reduction in their number. In the control animals, after the clamps had been removed, the eosinophils dissappeared almost entirely from the blood. Apparently, in the animals with transplanted adrenals, the adrenal cortex is functionally deficient.

The facts described above show that during the month following autografting, despite the structural recovery of the adrenals [3], the animals still showed a reduced resistance to trauma, on account of functional adrenal cortical insufficiency. Indeed, in these animals the trauma did not produce such a marked drop in the number of eosinophils as it did in the control experiments.

As we showed in our work with D. Ya. Shurygin [3], there had been an almost complete restoration of the mineral corticoid function of the adrenal cortex by the time the trauma had been inflicted, as was shown by the return of the salt content of the urine and plasma to normal. The reduced resistance to trauma must therefore be associated with insufficiency of the glucocorticoid adrenocortical function. The same conclusion is indicated by results obtained in experiments on the effect on shock of mineral and glucocorticoids. In practice it was found that treatment of shock with deoxycorticosterone acetate is without effect, whereas the hypophyseal adrenocorticotropic hormone and cortisone have a markedly favorable effect [2].

It should be noted that in animals with transplanted adrenals, during shock there is a marked hypothermia, a thickening of the blood, and an intense leucocytosis.

SUMMARY

The effect of mineral and glucocorticoid adrenocortical function on the pathology of trauma was studied in rats with autotransplanted glands.

Studies were made of behavior, survival time, temperature and blood.

It was found that during the first month after autografting, despite the recovery of the cortical structure of the

adrenals, the resistance of the animals to trauma was reduced; this effect may be attributed to adrenocortical glucosteroid failure.

The shocked autografted animals also showed pronounced hypothermia, a thickening of the blood, and an intense leucocytosis.

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THE EFFECT OF ADRENOCORTICOTROPIC HORMONE ON POST-MORTEM RIGIDITY

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In studying the effects of adrenalectomy, we found that it causes a considerable delay in the development of post-mortem rigidity. Here we have attempted to find which hormone it is whose absence in the adrenalectomized rats produces this effect.

METHOD

The experiments were carried out on 90 adult rats, all from the same nursery. The adrenalectomy was performed under ether anesthesia. After operation, for one week, 30 of the adrenalectomized animals received intraperitoneal injections either of a 0.5% solution in oil of 5 mg per 100 g body weight of cortisone and the same amount of deoxycorticosterone, or 5 units per 100 g of cortin. Forty of the adrenalectomized group received no hormone therapy after the operation. All the animals were given milk and tap water (without the addition of sodium chloride).

Two hours after the last injection, the rats were decapitated, and the rate of stiffening of the tail measured [2].

RESULTS

It can be seen from Fig. 1 that adrenalectomy delays post-mortem rigidity.

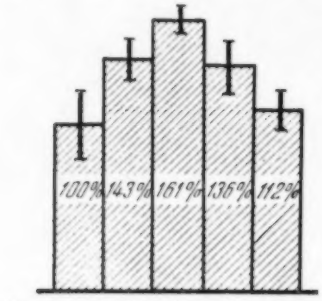

Fig. 1. The effect of adrenalectomy on the time of onset of post-mortem rigidity of skeletal muscle (time for the control rats taken as 100%).

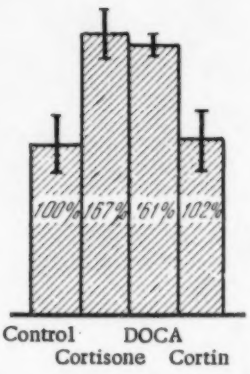

Fig. 2. The effect of adrenocortical hormones on post-mortem rigidity in adrenalectomized rats. Measurements made one week after adrenalectomy (time for the control rats taken as 100%).

The slowest development of rigidity occurred when the rats were killed two weeks after adrenalectomy. When killed after three weeks, rigidity developed somewhat more rapidly; after four weeks its onset occurred approximately at the same time as in the control groups. Evidently, 3–4 weeks after the operation sufficient corticosteroids are being produced [1].

The results indicate that corticoid insufficiency delays post-mortem rigidity. The original rate can be restored only by injecting preparations containing both mineral and glucocorticoids (cortin), and no effect is produced by injecting the hormones separately (Fig. 2).

In the light of these results, the delay in the onset of post-mortem rigidity in newborn animals, as discovered by L. I. Tank [2], may be attributed to an insufficient development of their hypophyseal-adrenal system [3,5]. Tank showed that at the age of 2-3 weeks, post-mortem rigidity develops at the same rate as in adult animals, indicating that the hypophyseal-adrenal system is fully functional [4].

SUMMARY

Post-mortem rigidity develops more slowly in adrenalectomized rats than in intact animals. Administration of cortin containing mineral and glucocorticoids equalizes the rate of onset of post-mortem rigidity in adrenalectomized and intact rats. These corticoids given separately produce no such effect. Delayed post-mortem rigidity in newborn rats (detected by L. I. Tank)is attributed by the author to the insufficient development of their hypophyseal-adrenal system.

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THE EFFECT OF CORTISONE ON THE COURSE OF EXPERIMENTAL STAPHYLOCOCCAL INFECTION

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We have shown previously [2,3] that the introduction of staphylococci into the nose of mice does not lead to any infectious process. In many cases, by various local actions on pulmonary tissue it was possible to produce a staphylococcal pneumonia which impaired the activity of the alveolar phagocytes and the drainage function of the bronchii. However, it is known that not only the local conditions but also the responsiveness of the organism as a whole is important in determining the development of an infection.

The aim of the present work has been to produce an experimental staphylococcal infection in mice by suppressing natural resistance by means of cortisone. This particular preparation was used because the glucosteroids are the most powerful means of reducing inflammatory reactions.

A number of investigations [6,7,9,12–16] have demonstrated the action of cortisone in suppressing the leucocytic reaction. According to Gell and Hinde [10], Crabbe [8], Nicol, Snell and Bilbey [17], cortisone suppresses the phagocytic activity of the cells of the reticuloendothelial system, although Z. A. Éruganova and L. S. Ageeva [5] found only a reduction in the assimilative power of these cells.

Very few investigations have been made of the effect of cortisone on the course of an experimental staphylococcal infection. Lichtwiz, Hioco and Thiery [14], in experiments with subcutaneous infection, observed that cortisone reduced the size of an abscess, and suppressed both the development of new vessels and the development of a fibrous capsule.

METHOD

Experiments on 353 white mice were made to determine the effect of cortisone on the course of a staphylococcal infection.

In the first experiment, the animals received 2.5 mg of cortisone acetate subcutaneously. After three hours, they were infected intranasally, under light ether anesthesia, with a suspension of 300 million staphylococci per mouse. The bacterial strain used had been obtained from an abscess 1 ½ months before the experiments were carried out, and possessed the typical staphylococcal pathogenic properties. For 69 of the mice the cortisone used was prepared by the French firm Rusel, and for 23, a brand made by the American firm Merck; the 52 control mice were infected without previous treatment. In addition, a study was made of the organs of 25 white mice which had received a single injection of cortisone and had not been infected; they were killed 1, 2, and 4 days after receiving the injection.

In a second experiment on 47 mice which had been prepared by a single injection of cortisone (Rusel) and 39 control animals, the intranasal staphylococcal dose was increased to 1.5 billion.

In a third experiment 98 mice received 2.5 mg of cortisone subcutaneously daily for 6 days; 11 of them died after 4–6 injections. Of the remaining 87 mice, 34 were injected with 300 million staphylococci 24 hours after the last injection, while the remainder were infected after 1 and after 5 days.

In the first and in the third experiments both the experimental and the control animals which had been infected with staphylococci were killed with ether after 1,3,6,12 and 18 hours and after 1,2,3,4 and 5 days. In the second experiment, where many animals died, a study was made of those which died in the first two days, while the remainder were killed 5 days after being infected.

Histological preparations of pulmonary tissue and of certain other organs were stained with hematoxylin-eosin, by Gram-Weigert and by Goldman's Sudan-alphanaphthol. In addition, studies were made of smear preparations which in some cases were taken from the cut surface of the lung and stained in azure-eosin.

RESULTS

In the first experiment, after the intranasal injection of 300 million staphylococci, those reaching the lung were rapidly eliminated, due to the activity of the alveolar phagocytes and the granular leucocytes which migrated into the alveoli. No infection occurred.

In mice which had been prepared by an injection of cortisone, the staphylococci were phagocytosed by macrophages, but the action was not vigorous. Thus, whereas in the control animals, after 6 hours, 80-99% of all the staphylococci had been phagocytosed, in the experimental group, only 23-69% had been eliminated in this way. There was a still more marked difference in the leucocytic reaction, which in the experimental mice was much less well shown in the early stages. In them, the leucocytes accumulated in various quantities in the capillaries, and most frequently in the region traversed by the alveoli, while the lumen of the alveoli contained practically no staphylococci, and practically none were captured there. In these animals there was a striking multiplication of the bacteria, and after 6 hours their number had greatly increased. Later, the process took different directions in the different animals.

In some of the mice which had been prepared by cortisone, the leucocytic reaction was enhanced; polynuclears, filling some of the alveoli, phagocytosed and destroyed the bacteria. One to two days after the beginning of the experiment, the number of staphylococci had been greatly reduced, and later, on the 4th and 5th days, they had disappeared almost completely from the pulmonary tissue. Thus, after a short period of multiplication, changes occurred which were approximately the same as in the control group, and the only difference was that they lasted for a longer time.

In addition, in 10 out of the 35 mice which had been prepared by a cortisone injection and killed 6-24 hours after receiving the staphylococci, the bacteria grew in the pulmonary tissue to form colonies, and abscesses developed (see figure). This focal multiplication of bacteria occurred in 6 of the 11 cases in which the American cortisone was used, but in only 4 out of 24 animals which received the French cortisone.

In the second experiment, when the dose of staphylococci was increased to 1.5 billion, in all the animals which had received a cortisone injection a severe infection occurred with an explosive multiplication of bacteria in the lungs. In the two days before the experiment, 39 of the 47 mice died, and in 4 of the remaining 10 which

Abscess formed in the lung of a mouse containing a large number of staphylococcal colonies in the center. Animal previously given a cortisone injection. Mouse killed 24 hours after injection. Gram-Weigert stain. Magnification 50 ×.

were killed after 5 days, there were large abscesses in the lungs and a fibrinous necrotic pleuritis and pericarditis. Of the 39 control animals, only 3 died, and it was only in them that the staphylococci were found to have multiplied in the pulmonary tissue.

In the third experiment, where we gave repeated injections of cortisone, many of the mice developed spontaneous infections of the respiratory passages due to autogenous infection with fungi, as well as an infectious hepatitis. Nevertheless, these animals readily tolerated the introduction of 300 million staphylococci intranasally, and no infection occurred.

Thus, in our experiments, it was only a single preliminay injection of cortisone which enabled a staphylococcal infection to take place in animals in which the intrapulmonary introduction of these bacteria is usually without effect. Under the influence of cortisone, in addition to some reduction in the phagocytic activity of the pulmonary macrophages, there was a marked delay in the leucocytic reaction occurring in the first few hours after introducing the staphylococci. On this account, in all the animals which had been prepared by a cortisone injection, the staphylococci multiplied in the lungs. However, subsequently in some animals the growth of the bacteria ceased. This effect is probably due to a restoration of phagocytic activity, although in many of the mice, and particularly in those which had received the American cortisone, there was a focal growth of staphylococcal colonies leading to the formation and delimitation of abscesses.*

Increasing the number of bacteria used caused most of the cortisone-treated animals to die, and serious pulmonary damage occurred in some of the survivors. However, under these conditions some mice recovered from the infection. Thus, cortisone in the dose used by us exerted a short but well marked action by destroying the phagocytic activity of the macrophages, and by preventing the migration of the polymorphs, which under normal conditions play an active part in the destruction of the staphylococci.

SUMMARY

Mice which are normally resistant to staphylococci will usually become infected if a preliminary injection of cortisone is given. In addition to producing some reduction of the phagocytic activity of the macrophages of the lungs, cortisone delays the leucocytic response occurring within the first few hours after the infection. The effect is more pronounced with large doses of bacteria. American cortisone is more effective in this respect than the French preparation. Prolonged use of cortisone has no effect on the development of staphylococcal infection. Infection of the lungs by fungi occurs commonly in animals so treated.

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* Other investigators [1,4] have also noticed the difference in the action of the American Merck cortisone and that of the English and French varieties.

PROTEIN COMPOSITION OF THE BLOOD SERUM IN EXPERIMENTAL HYPERTENSION INDUCED BY A PARTIAL CONSTRICTION OF THE LUMEN OF V. PORTA AND ISCHEMIZATION OF THE LIVER

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In recent years, through clinical observations by many authors [2,3,9], the development of hypertension has been established in patients suffering from thrombosis and embolism of the vessels of the abdominal cavity, as well as upon the impairment of local circulation in the liver and other pathological processes in the organs of the abdominal cavity.

We established in previous experiments on dogs [6,7] that in acute and partial constriction of the lumen of the portal vein and ischemization of the liver caused by the application of constricting ligatures a prolonged and marked hypertension develops.

The present work is devoted to the study of changes of the protein fractions of the blood serum in dogs which had been subjected to the above-stated intervention.

METHOD

Studies were carried out on seven dogs in chronic experiments which lasted from 2 to 17 months. We performed a total of 48 refractometric and electrophoretic determinations of the composition of blood serum proteins; of these 15 were controls and 33 were carried out during the process of development of hypertension induced by the above-stated intervention. As controls, we used the indexes of the initial determinations performed twice on each dog prior to the development of hypertension, and in one dog prior to and following the operation of a laparotomy, but without constricting the portal vein and ischemization of the liver.

Blood specimens were obtained from the femoral vein. Directly after obtaining the specimen we prepared the serum in which we determined the protein content by means of a Pulfrich refractometer and electrophoresis on paper. The electrophoresis lasted 18 hours in a veronal buffer at pH 8.6, voltage 5 v/cm, and current strength of 0.3 ma on 1 cm of the cross section. The electrophoregrams were stained with bromophenol blue in an acid bichloride of mercury solution and washed off with a 1% solution of acetic acid. The quantitative evaluation of the electrophoregram was made by means of a densitometer. The densitograms were additionally checked with electrophoregrams and cut into separate pieces, each corresponding to a definite protein fraction. The pieces of paper were weighed on analytical scales, following which the percentage ratio was calculated and, taking into account the refractometric data, the content of each protein fraction was calculated in gram-percent.

RESULTS

It can be seen in the composite table of changes in the indices of protein fractions that the total protein quantity in the blood serum of normal dogs constituted 6.8% on the average (fluctuation range 6-7%); of this, 3 gm% is albumins (fluctuation range 2.4-3.3%) and 3.8 gm% is globulins (fluctuation range 3.3 to 4.2 gm%). The albumin-globulin coefficient for normal serum is 0.8.

The α_1-globulin content equals 0.7 gm% (fluctuation range 0.4 to 1.0 gm%), α_2-globulins - 0.9 gm% (fluctuation range 0.5 to 1.4 gm%), β-globulins - 1.1 gm% (fluctuation range 0.5 to 2.0 gm%), γ-globulins - 1 gm% (fluctuation range 0.4 to 1.4 gm%). The data obtained in our experiments for normal serum coincide with the data in the literature [4,5].

It is seen in Table 1 that the changes in protein content are characterized by an increase of the total quantity of proteins of the blood serum by 0.2 gm% on the average. The most marked increase was noted in globulins

TABLE 1. Changes in the Quantity of Serum Proteins, Arterial Pressure, and Pulse in Norm and in Hypertension in Dogs

Dog's name	Type of operation	Examination date	Arterial pressure, mm Hg	Pulse rate per min	Amt. serum protein found (gm %)	Serum protein fraction content, gm%					
						albumins	globulins				
							total	α_1	α_2	β	γ
Norka		30/V 1957	135	93	7,0	2,8	4,2	1,0	1,4	0,9	0,9
		19/VI 1957	138	98	7,0	3,1	3,9	0,6	1,0	1,1	1,2
	Control laparotomy	1/XI 1957	130	94							
		11/XI 1957	134	91	6,8	2,6	4,0	0,5	1,0	1,5	1,0
Taiga		30/V 1957	118	70	6,8	3,3	3,5	0,5	1,2	0,5	1,3
	Constriction of portal vein lumen	5/VI 1957	126	74	6,9	3,2	3,7	0,6	1,0	0,7	1,4
		19/VI 1957	158	92	7,7	2,7	5,0	0,4	2,3	2,3	
		29/VI 1957	181	90	7,5	3,0	4,5	0,6	1,6	0,8	1,5
Dzhuba		30/V 1957	141	102	7,1	3,4	3,7	1,0	1,0	0,8	0,9
	Constriction of portal vein lumen	5/VI 1957	146	110	7,0	3,0	4,0	0,7	1,0	1,3	1,0
		19/VI 1957	185	120	7,8	3,7	4,2	0,4	1,1	0,9	1,8
		29/VI 1957	193	122	7,5	3,1	4,4	0,5	0,8	1,7	1,4
		11/XI 1957	236	136	7,7	3,0	4,0	0,5	1,5	1,4	0,6
		26/III 1958	170	108	7,6	2,7	4,9	0,7	1,1	1,9	1,2
		14/V 1958	160	92	6,8	2,8	4,0	0,6	0,8	1,5	1,1
		26/V 1958	177	102	7,0	2,6	4,4	0,7	1,2	1,6	0,9
		5/VI 1958	170	104	7,2	2,7	4,5	0,6	1,3	1,3	1,3
	Ischemization of the liver	17/VI 1958	161	92	7,0	2,6	4,4	0,9	0,9	1,3	1,3
		29/VI 1958	213	118	6,8	2,5	4,3	0,6	1,2	2,5	
Sharik		30/V 1957	120	88	7,0	3,0	4,0	0,5	0,5	2,0	1,0
	Ischemization of the liver	5/VI 1957	126	82	7,0	3,0	4,0	0,9	0,7	1,3	1,1
		13/VI 1957									
		19/VI 1957	171	108	7,4	2,9	4,5	6,6	1,1	1,7	1,1
		29/VI 1957	175	108	7,4	2,9	4,5	0,5	1,1	1,7	1,12
		11/XI 1957	142	98	7,4	2,6	4,82	0,6	1,0	3,22	
	Ischemization of the liver	28/XI 1957									
		26/III 1958	141	102	6,7	2,6	4,16	0,5	1,1	1,4	1,16
		14/V 1958	138	86	6,5	2,4	4,14	0,6	1,0	1,5	1,04
	Constriction of portal vein lumen	19/V 1958	130	92	6,4	2,3	4,1	0,6	1,0	0,8	1,7
		22/V 1958	170	112	7,2	2,3	4,9	0,6	1,4	1,6	1,3
		26/V 1958	183	120	6,75	2,1	4,65	0,6	1,2	1,7	1,15
		30/V 1958	164	120	7,5	2,6	4,9	0,5	1,3	2,1	1,0
		2/VI 1958	178	120	7,1	2,2	4,9	0,7	2,3	1,9	
Barsik		24/V 1957	129	80	6,7	3,4	3,4	0,7	1,3	1,0	0,4
		30/V 1957	128	62	6,6	3,3	3,3	0,6	0,9	1,1	0,7
	Constriction of portal vein lumen	1/VI 1957									
		5/VI 1957	188	100	7,0	2,4	4,6	0,4	1,3	1,7	1,2
		19/VI 1957	197	102	7,1	2,4	4,7	1,3	1,3	0,9	1,2
		29/VI 1957	199	108	7,0	2,4	4,6	0,45	1,25	2,0	0,9
Kazbek		5/IV 1958	122		6,8	2,8	4,0	0,4	1,0	1,6	1,0
	Constriction of portal vein lumen	14/V 1958	113	80	6,4	2,8	3,6	0,7	0,8	1,3	0,8
		19/V 1958	188	102	7,0	2,3	4,7	0,8	0,9	1,9	1,1
		26/V 1958	194	100	7,2	2,5	4,7	0,5	1,1	1,5	1,5
		30/V 1958	204	104	7,3	2,5	4,8	1,5	1,8	1,0	0,5
Buyan		14/V 1958	121	80	6,9	2,3	4,15	0,5	0,9	1,6	1,25
	Constriction of portal vein lumen	19/V 1958	121	86	6,9	2,8	4,15	0,94	0,7	1,45	0,86
		22/V 1958	176	102	6,6	2,5	4,1	0,6	0,9	1,7	0,9
		26/V 1958	178	104	6,3	2,4	3,9	0,6	1,0	1,6	0,7
		30/V 1958	184	106	5,9	2,1	3,8	0,7	0,8	1,4	0,9
		2/VI 1958	165	114	6,0	2,2	4,76	0,6	1,2	1,3	1,66
	Ischemization of the liver	17/VI 1958	133	80	6,6	2,4	4,2	1,3	0,9	0,8	1,2
		29/VI 1958	156	82	6,6	3,0	3,6	0,7	1,3	1,6	

TABLE 2. Statistical Analysis of the Data Cited in Table 1

Index	Symbol	Serum protein content, gm%	Serum protein fraction content, gm%					
			globulins					
			glo-bulins	albu-mins	α_1	α_2	β	γ
Arithmetical mean in norm	M_1	6,8	3,0	3,8	0,7	0,9	1,1	1,0
Arithmetical mean in hypertension	M_2	7,0	2,6	4,4	0,6	1,1	1,5	1,2
Mean square deviation in norm	$\sigma_1\pm$	0,355	0,251	0,295	0,277	0,232	0,408	0,427
Mean square deviation in hypertension	$\sigma_2\pm$	0,679	0,316	0,358	0,25	0,39	0,379	0,320
Standard deviation in norm	$m_1\pm$	0,094	0,067	0,079	0,072	0,062	0,136	0,066
Standard deviation in hypertension	$m_2\pm$	0,118	0,05	0,062	0,045	0,067	0,074	0,062
Index of the difference of the data significance	T—	1,3	4,7	6,0	2,5	2,2	2,7	2,2
Difference probability	P—	0,2	0,01	0,001	0,05	0,05	0,05	0,05

(by 0.6 gm% on the average, fluctuation range 3.6-5.0 gm%), whereas the quantity of albumins showed a regular decrease (by 0.4 gm% on the average, fluctuation range 3.1-2.1 gm%).

Changes in separate globulin fractions were not uniform in their magnitude. For instance, the content of α_1-globulins changed least of all, whether in the direction of an increase or decrease, and in one dog no changes were observed. The content of all other globulin fractions in all dogs showed a regular increase: α_2-globulins by 0.2 gm% on the average (fluctuation range 0.8-2.5 gm%), β-globulins by 0.4 gm% (fluctuation range 0.8-2.1 gm%) and γ-globulins by 0.2 gm% (fluctuation range 0.5-1.8 gm%). Statistical analysis of the data obtained demonstrated their reliability (Table 2).

The data in the literature and the results of our experiments prove convincingly that the change in α_2-globulin content takes place in various deseases and various influences which are accompanied by the "stress" state according to Selye. On this basis we may assume that the changes in the α_2-globulin content cannot be placed in strict dependence on the development of hypertension only. In the process of development of the hypertension model in our experiments, as well as in renal-ischemic hypertension as per Page and Lewis [11] and M. Ya. Khodas [10], definite changes have been observed in the direction of an increased content of β- and γ-globulins. In addition, S. V. Belyakova [1] reports that in hypertensive patients an incease of the quantity of fibrinogen takes place.

In the literature devoted to the problem of allergy, the γ-globulin fraction of serum proteins is referred to as the material substrate of various antibodies, including autocytolysins. It is a known fact that nephrocytolysins represent the pathogenetic factor in the development of glomerulonephritis and marked hypertension. This fact has been also corroborated experimentally. The increase in the amount of γ-globulins which we have observed in all experimental dogs leads us to the assumption of their possible pathogenetic role in the development of hypertension.

SUMMARY

Protein composition of the blood serum was examined in 7 dogs prior to and after the development of hypertension caused by a partial constriction of the portial vein lumen and ischemization of the liver. A total of 48 experiments were carried out; 15 of these were control and 33 with hypertension. As established, an increase in the blood serum globulins and a reduction of albumins were seen in all the animals with hypertension. A rise of the β- and γ-globulin fractions was noted; there was also a change in the direction of increase or reduction of α_1-globulins. On the basis of literature data and personal experiments, a conclusion is drawn that the change in the serum α_2-globulin content is not specific for the hypertensive states.

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All abbreviations of periodicals in the above bibliography are letter-by-letter transliterations of the abbreviations as given in the original Russian journal. *Some or all of this periodical literature may well be available in English translation.* A complete list of the cover-to-cover English translations appears at the back of this issue.

THE EFFECT OF IONIZING RADIATION ON THE CONTENT OF FREE AND BOUND ACETYLCHOLINE IN THE LIVER AND BRAIN

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(Presented by Active Member AMN SSSR N. A. Kraevskii
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Analysis of the mechanisms of development of radiation affections indicates that in the pathogenesis of radiation sickness various changes in the neuro-humoral regulatory processes play a prominent part [1,10,12-15]. At the same time, considerable disturbances also originate in the metabolism of local origin. Therefore, of considerable interest is the effect of ionizing radiation on the metabolism of those natural chemical agents of high biological activity which directly participate in the neuro-humoral and local regulation of biochemical processes.

Acetylcholine [ACh] attracted our attention as one of these substances. On the one hand, its effect on the organism is directly connected with the activity of numerous neural elements. This fact determines the immense effect of ACh in the regulation of the tonus of blood vessels, cardiac rhythm, dynamics of the gastrointestinal tract, functions of the glands of internal and external secretion, muscular activity, and the activity of many central neural apparatuses. On the other hand, ACh in larger or smaller quantities is formed in the tissues of the liver, muscles, placenta which has no neural elements, etc. ACh as a "local hormone" apparently plays an important role in the regulation of the physicochemical state of various interphase borders within the cells by affecting their polarization and permeability. In this connection, the property of ACh of enhancing the metabolism of phospholipids, possibly as the result of disintegration of lipoproteid complexes, is of great importance [29,30]. It exerts considerable and diverse (depending on conditions) effect on the activity of a number of most important enzymic systems [2,4,6,7]. It should be pointed out also that from the specific radiobiological point of view the effect of ACh on sulfhydryl groups' activity is highly important [2,3,5,8,16,19], as well as on the oxidation processes [2,27,28,31] and, in particular, its property of enhancing the resistance of the organism to hypoxia [25,33,34].

Experiments of many authors elicited in radiation injuries various deviations of ACh metabolism from norm. In the majority of cases, during the remote period after irradiation a reduction of cholinesterase activity in the blood serum [11,17,18,26,32], as well as in some tissues [21-24,26], has been observed. Prior to this reduction, wave-like fluctuations of cholinesterase activity were observed, with rises during the early stages after irradiation [9,17,18,20]. A detailed investigation of ACh metabolism following external (x-ray) and internal (polonium and uranium fission products effects) irradiation was carried out on rats by E. N. Petrovnina [17]. She investigated, simultaneously with the determination of general cholinesterase activity in a number of tissues, also the changes in the total ACh content in them, as well as the cholinacetylase activity of the brain. She established, in particular, that the total ACh content in the tissues during the early stages of radiation sickness had been reduced and that, later, the tendency toward its increase was manifested.

The interpretation of the available data is hampered by the fact that in the overwhelming number of investigations only the total cholinesterase activity and the total ACh content (or of the free ACh only) were determined. We were intersted in the problem of the bound ACh. Apparently, bound ACh being impervious to the attack of cholinesterases is not biologically passive precisely on this account. By combining at various periods of time with biological objects, acetylcholine changes their properties, thus manifesting a "trigger" or "trophic" activity.

In the present series of investigations we carried out determinations of the free (conditionally ?) and bound ACh in the tissues of the liver and brain of white rats following total external radiation of Co^{60} gamma-rays in a dose of 800 r within 2,24, and 48 hours after irradiation

METHOD

Quite a number of methods of determination of bound ACh have been suggested consisting of denaturation of proteins and destruction of lipid formations. We decided on the heating method without introducing into the tests any other substances except cholinesterase inhibitors. For the determination of ACh we employed as a test object the dorsal muscle of a leech. Its reaction (contraction) was investigated by testing extracts where ACh had been specif-

Content of Free and Bound ACh in the Liver and Brain of White Rats following Total Irradiation (in microgram%)

Liver		Brain	
free	bound	free	bound
acetylcholine		acetylcholine	
Norm			
0,04	0,62	2,2	84
0,06	0,05	15,4	49
0,06	0,05	4,4	78
0,17	4,78	1,1	49
0,46	0,45	5,9	94
0,23	0,72	8,4	71
		1,1	72
0,44	4,29	28,6	70
0,06	0,05	15,4	66
0,11	0,99	29,7	39
		9,9	83
Average			
0,18± ±0,06	1,33± ±0,61	11,1±3,1	69±5
Within 2 hr after irradiation			
traces	4,40	16,5	79
»	traces	11,0	76
0,04	0,06	5,5	76
0,99	1,76	10,7	97
traces	5,50	16,5	83
»	traces	5,5	60
»	0,65	2,2	34
»	0,10		
Average	—	9,7±2,1	72±8
Within 24 hr after irradiation			
0,01	0,10	7,9	25
0,03	2,39	9,9	100
0,03	2,17	6,6	67
0,04	0,05	5,5	79
none	0,06	28,1	51
»	0,29	4,9	108
0,04	0,05	32,5	41
none	0,08	3,3	97
0,04	4,03	1,1	34
		2,8	74
Average	—	10,3±3,5	68±9
Within 48 hr after irradiation			
traces	0,40		
0,10	2,40	8,8	68
0,02	0,08	0,1	44
0,30	11,10*	1,1	86
0,10	0,30	0,9	71
0,60	0,50	16,5	59
Average			
0,22± ±0,027	0,74± ±0,35	5,5±3,1	66±7

*These data were not included in the average.

ically destroyed with a cholinesterase preparation. The tested tissue samples were homogenized and extracted with a Ringer solution (with pH 7.6) by heating (for the determination of bound ACh) or in cold (for the free ACh). The obtained extract was placed in a container with a preparation of the dorsal muscle of a leech, and the extent of its contraction was tested kymographically and compared with the extent of contraction induced by an ACh solution of known concentration. The quantity of bound ACh was determined according to the difference between the total ACh content and the free ACh content. Irradiation was carried out with gamma-rays an EGO-2 apparatus by an 800 r dose at a dose strength of 460 r/min.

The obtained data indicate that in norm the content of free and bound ACh in the tissues of the liver and brain varies considerably. In regard to the total content of any given fraction, the ACh quantity in the brain exceeds by several ten-folds that of the liver. In view of the considerable divergence between the data of individual determinations we cited in the table the entire experimental material.

As seen in the table, the free ACh content of the liver varied from 0.04 to 0.46 microgram% and averaged 0.18 microgram%. Within two hours after irradiation, almost every test revealed only traces of free ACh; in one case, on the contrary, its quantity turned out to be approximately twice as high as the normal maximum. Subsequently, within 24 hours after irradiation the free ACh content in the liver tissue became accessible for quantitative determination in a number of cases, but it was still very low; within 48 hours after irradiation its quantity increased and reached almost normal levels.

The content of bound ACh of the liver in norm was several times higher than that of free ACh; with variations from 0.05 to 4.78 microgram%, it constituted 1.33 microgram% on the average. Within 2 hours after irradiation the quantity of bound ACh varied even more markedly than in norm—from traces to 5.50 microgram%. The data obtained within 24 and 48 hours after irradiation proved to be on the whole close to those in norm; we should mention only one very high figure-11.10 microgram%-elicited in one case 48 hours after irradiation.

In norm the free ACh content of the brain (see table) fluctuated between 1.1 and 29.7 microgram%, or 11 microgram% on the average. Within 2 and 24 hours after irradiation, no appreciable deviations from norm were observed. Within 48 hours after irradiation, a certain reduction of the free ACh content was also found in the cerebral tissue. The amount of bound ACh inthe cerebral tissue was also several times higher than free ACh. It constituted about 70 microgram% on the average. As shown by the above-cited data, no changes in the content of bound ACh in the cerebral tissue of rats were observed within 2,24, and 48 hours after irradiation.

Thus, the early stages of acute radiation sickness in rats are characterized by a considerable reduction (almost disappearance) of the free ACh content of the liver within 2 hours after irradiation, and its gradual normalization within 48 hours after irradiation. In the brain the free ACh quantity is also reduced, but this occurs only within 48 hours following irradiation and to a relatively small extent. The content of bound ACh, while there is a certain increase of the range of its fluctuations in the liver, remains on the whole fairly stable, especially in the brain.

SUMMARY

In testing on leech dorsal muscle the authors determined the contents of free (conditionally) and bound acetylcholine (ACh) in the liver and brain tissues of rats both in normal conditions and following γ-ray irradiation in a dose of 800 r. As demonstrated, the content of free and bound ACh was several times greater in the brain than in the liver. Considerable individual variations were noted. In 2 hours after the irradiation the content of free ACh in the liver decreased to only traces. Its initial level was gradually restored by the 2nd post-irradiation day. The content of free ACh in the brain dropped too, although to a much lesser extent. The concentration of bound ACh remained rather stable in the brain of irradiated animals, whereas the range of its variations in the liver had somewhat increased.

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COMPARATIVE HISTOCHEMICAL DATA ON GLYCOGEN CHANGES IN X-RAY AND STRONTIUM-90 RADIATION SICKNESS

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In our previous investigations [5] we demonstrated that, under conditions of acute radiation sickness induced via x-rays (800 r), a serious impairment takes place of the glycogen content in the liver, cardiac muscle, lungs, and other organs of the irradiated animals. Thus, within the first few hours after irradiation a marked reduction of the liver glycogen is observed and an increase of its content in the cells of cardiac muscles. During the latter stages, on the contrary, a certain restoration of the glycogen content of the liver and cardiac muscle is noted. However, during the entire acute period of the disease the glycogen content remains reduced. Only in animals who had recovered from the acute stage of the disease did glycogen content in the organs gradually reach its normal level.

These observations fully conform with the data of various clinical, pathologoanatomical and biochemical investigations during various periods of the acute radiation sickness.

It could be assumed that under the effect of incorporated radioactive substances the character of corresponding histochemical observations will differ from the previously described ones, since there is a definite pecularity in the clinical and pathologoanatomical pictures of this type of radiation injury.

On this basis, we carried out histochemical studies of glycogen and alkaline glycerophosphatase in the liver, cardiac muscle and lungs of rabbits during the early stages of chronic radiation sickness induced by the introduction of strontium-90.

METHOD

A total of 33 rabbits was used in the experiments. Of these, 21 received intravenously strontium-90 in a 0.5 microcuries / gm dose. Twelve healthy rabbits served as control. The rabbits were killed within 6 hours and 1,3,5,7,10,15 and 20 days from the moment of strontium-90 administration. Determination of glycogen was made according to the A. L. Shabadash method [14], and alkaline glycerophosphatase by the Gomori method. We also determined the amount of energy absorbed in the liver, cardiac muscle and lungs.

RESULTS

In all experimental animals the initial stage of the disease (up to 20 days) proceeded with marked phenomena of acute radiation sickness, though with a less expressed leucopenia than in the case of x-ray irradiation. The number of leucocytes per cubic mm of blood was reduced to 2000-3000.

Thus, we had sufficient reason. to evaluate the character of glycogen changes in the investigated organs in acute radiation sickness caused by total x-ray irradiation and during the acute period of a chronic radiation sickness caused by the incorporation of strontium-90.

We took into account that strontium-90 (beta-emitter) is accumulated basically in the bones very soon after its incorporation. Therefore, all pathologoanatomical changes in the liver, cardiac muscle and lungs which take place following administration of strontium-90 should be regarded mainly as the result of general disturbances caused by the presence of a radioactive substance in the organism.

The detected morphological changes in the liver, cardiac muscle and lungs manifested themselves in phenomena of acute impairment of the hemodynamics, increased permeability of vascular walls, and to a greater or lesser extent in pronounced dystrophic changes in the parenchymatous cells and interstitial tissue. These changes have been sufficiently described in detail in the literature [3,8,11] and we shall therefore not dwell on them.

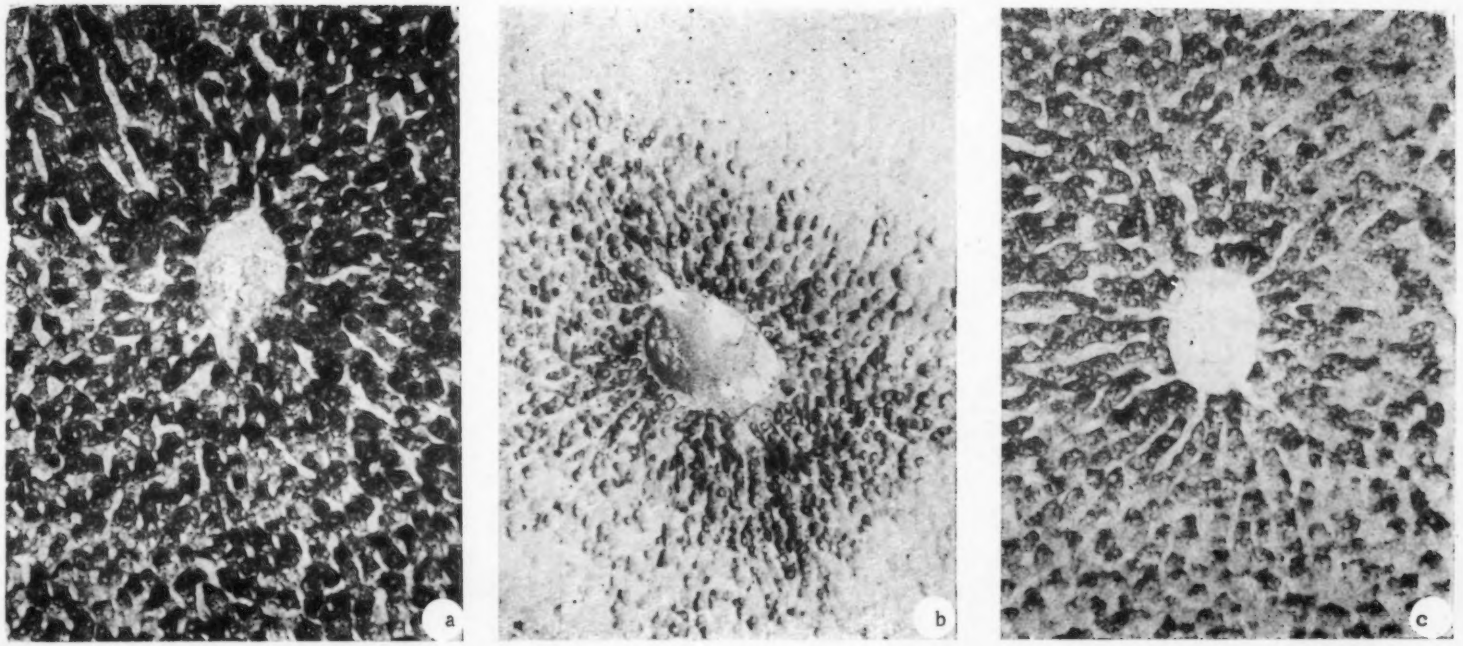

Fig. 1. Changes in the glycogen content in the liver of healthy and irradiated animals. a) Control (healthy rabbit); b) 6 hours after irradiation with x-rays in an 800 r dose; c) 6 hours after the introduction of strontium-90 in the amount of 0.5 microcuries/gm. Staining by Shabadash method. Magnification obj. 10×, ocular 10×.

As regards glycogen changes, the data of histochemical observations during the first few hours following the introduction of a radioactive substance proved to be non-uniform. Despite repeated experimental check-ups, some animals showed a reduced glycogen content, others its increase, and in some instances its content did not differ from control. In the healthy control animals no such fluctuations of the glycogen content were observed. Analogously, the activity of alkaline glycerophosphatase varied. In some cases, despite an increased activity of the enzyme, the glycogen content of the liver showed no changes. In the cardiac muscle and lungs of all animals, parallel with the reduced glycogen content an increased activity of alkaline glycerophosphatase was noted. All this indicates the different character of the initial reaction in response to the introduction of strontium-90. This fact is apparently not accidental, since it fully conforms with the observations of other investigators who have also noted the non-uniform character of the derangement of various organic functions during the early stages following the effect of radioactive substances [10,13].

The nature of distribution of glycogen during the first hours and days following strontium-90 incorporation differed from its distribution after x-ray irradiation. This was most clearly observed in the liver. Whereas following x-ray irradiation glycogen could be elicited only in the cells of the central part of lobules, a uniform distribution of glycogen over the entire lobule was observed following strontium-90 administration (Fig. 1). In the first case, the rapid decrease of glycogen is, possibly, connected basically with the reflex shifts in the carbohydrate metabolism; in the second case a gradual development of deeper disturbances of intracellular metabolism takes place. This is partially attested to by the increased activity of alkaline glycerophosphatase.

The above-stated equally refers to the changed content of glycogen also in other organs. Thus, we observed no initial increase of glycogen content in the cardiac muscle, which is generally observed under conditions of x-ray irradiation. In the lungs also a definite reduction of glycogen content was observed in the macrophages and hyaline cartilages of the bronchi.

An increased content of glycogen in the investigated organs in some animals can be connected during the early stages following the introduction of strontium-90 with its redistribution as well as with liberation of the so-called structural glycogen not elicited under ordinary conditions via our method. The latter explanation, in our opinion, seems to be more likely.

On the 2nd-3rd day and during the latter stages of the disease, the glycogen content of the liver, cardiac muscle and lungs was considerably lower than in control animals. The reduction was observed in all animals, and it was more marked as the disease progressed (Fig. 2). However, the glycogen distribution in the tissues now had a uniform character similar to the one in acute radiation sickness induced with x-ray irradiation. The only difference was that in the case of strontium-90 no restoration of glycogen content had taken place toward the end of the observation period (15th-20th day). Simultaneously, a considerable increase of alkaline glycorophosphatase activity was observed.

In summarizing the above data, it is important to point out the substantial difference in the character of changes of the glycogen content in acute radiation sickness induced by x-rays and in the acute period of the chronic radiation sickness induced by the internal administration of strontium-90. In the first instance, the microscopic picture of glycogen distribution has much in common with the picture observed during a state of shock. It can be assumed, therefore, that the decrease of glycogen during the early stages of acute radiation sickness is connected mainly with acute functional shifts originating under the effect of external irradiation. In the second instance, the glycogen change takes place in all parenchymatous cells and is characterized by a gradual reduction of its content as the disease progresses. This type of change in the distribution of glycogen corresponds to the nature of the effect of incorporated radioactive substances on tissues. Whereas upon a single external irradiation there is a one-stage absorption of a very large quantity of radiation energy, in the affection caused by incorporated substances there is a gradual accumulation of energy absorbed by the tissues (see table).

Analysis of the obtained data indicates that in some animals apparently the impairment of glycogen synthesis predominates, as a result of which we observed a uniform reduction of glycogen in all cells of the liver, cardiac muscle and lungs. In other animals, as a result of the predominance of phanerosis of the structural compounds which include glycogen, the latter is elicited in excessive amounts. Finally, at the early stages following the introduction of radioactive substances the glycogen content of the tissues may show no changes. This fact conforms with the data of a number of authors [1,2,19,21] who had also found a normal quantity or an increased glycogen content in the tissues in radiation sickness. In their opinion, this is due to the delay in the disintegration of glycogen and its synthesis from the products of increased disintegration of proteins and fats.

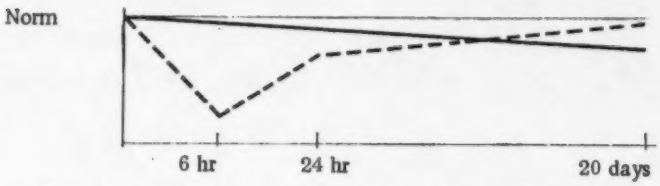

Fig. 2. Comparative diagram of the change in glycogen content in rabbits' liver under the effect of x-rays (----) and strontium-90 (——).

Quantity of Energy Absorbed(in rads)by the Tissues of the Liver, Lungs, and Cardiac Muscle under the Effect of Strontium-90 in a 0.5 microcurie/gm Dose

Organ	Time during which the energy absorption took place,days					
	1	2	3	5	10	20
Liver	0,015	0,023	0,026	0,034	0,064	0,11
Lungs	0,17	0,20	0,22	0,24	0,31	0,32
Cardiac muscle	0,02	0,05	0,053	0,071	0,10	0,15

One of the causes of the retarded splitting of glycogen may be the change in oxidation processes in the tissues of an irradiated organism [5,6,9]. As regards the reduction of the glycogen content at the height of the disease, one may assume that the mechanism of this phenomenon under conditions of external irradiation as well as after incorporation of radioactive substances is of an identical nature. Apparently, in both instances a profound disturbance of the metabolism takes place. However, in contrast to the opinion of a large number of authors [4,12,16,18,20,24,25] who think that the reduced glycogen content in the liver is connected with the insufficient absorption of carbohydrates from the intestines, there are data available at present which point to the leading role of inhibition of the glycogen-forming function of the liver in the impairment of glycogen metabolism [7].

Apparently, the cause of reduced glycogen content in tissues in radiation sickness is also the increased hydrolysis of the esters of phosphoric acid which are formed upon phosphorylation of monosaccharides in the process of their absorption from the intestinal canal and as a result of the phosphorolysis of glycogen. This is attested to in particular by the increased activity of alkaline glycerophosphatase. In this case an enhanced and premature disintegration takes place of compounds needed for the synthesis of glycogen and replenishment of the energetic and plastic reserves in the cells of an irradiated organism.

Thus, our data correspond to the observations of investigators who had observed a considerable reduction of glycogen in tissues following exposure to various sources of radiation [4,15,17,22,23]. Some divergence of our data from those of other authors can be, perhaps, explained on the one hand by the difference in the methods of investigation (histochemical and biochemical) and, on the other, by the non-uniform conditions of radiation action. As shown by our observations, the latter have a substantial effect on the investigation results.

SUMMARY

A histochemical study was made of the glycogen content in the liver, cardiac muscle and lungs of rabbits at the initial period of chronic radiation sickness provoked by the intravenous injection of strontium-90 in the dose of 0.5 μC per g of body weight. An essential difference was noted in the character of the initial changes of glycogen content in radiation sickness caused by the irradiation with x-rays and strontium-90. In the first case the changes in the glycogen content may be explained by the functional disturbances, whereas in the second—by more profound derangements in the carbohydrate metabolism. At the height of the disease the cause of reduced glycogen content is the same, and, according to the authors' data, is to a certain extent connected with the increased activity of alkaline glycerophosphatase.

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THE EFFECT OF IONIZING RADIATION ON ELECTROLYTE METABOLISM IN RATS

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The glucocorticoid function of the adrenal cortex is all important in connection with the action of ionizing radiation. Comparatively few works deal with the effect of ionizing radiation on mineral corticoid function. Indirect evidence on changes in this function following radiation has been produced by E. B. Pavlova and A. E. Rabkina [1] by giving a large water load; they found that on the second and particularly on the third and fourth days after the rats had been irradiated with the minimal absolute lethal dose of x-rays, the water was retained within the body, and diuresis was impaired.

Numerous reports [2-5] describe investigations into sodium and potassium metabolism in the tissues of irradiated animals, and the excretion of these electrolytes. However, the results are contradictory, probably because of different experimental conditions. The rate of formation and excretion of aldosterone, which is the hormone regulating mineral metabolism, may serve as an index of the state of mineral corticoid adrenal function after irradiation.

We have taken an initial step in this direction and have obtained results on the absorption of sodium in the intestine, the sodium and potassium content of the blood plasma, and the excretion of these electrolytes into the urine of irradiated animals.

METHOD

The experiments were performed on 250 male rats weighing 160-180 g. They were totally irradiated with a dose of 700 r from an RUM-11 apparatus. Irradiation conditions were as follows: current strength 15 ma, voltage 180 kv , focussing distance 40 cm , filter 0.5 mm Cu and 1 mm Al. The age of the animals ranged from 7 to 16 days. Both before and after irradiation, the animals were kept in glass metabolic cages with arrangements for the separate collection of urine and faeces. Collections were made before irradiation, and on the 1st, 3rd, 5th, and 7th days after irradiation. On the days when the urine and faeces were collected, the animals were not fed. Flame photometry* was used to estimate the sodium and potassium in faeces, urine, and plasma. An isotope method was used to measure the absorption of sodium from the intestine.

RESULTS

Table 1 shows the results of electrolyte excretion from the urine of the control and irradiated rats.

The average result for the sodim and potassium content of the urine excreted per day in healthy rats lay within the limits of figures published previously [6]. The amount of sodium and potassium excreted per day after irradiation deviates strongly from the normal; the sodium is much less, and the potassium much greater. The Na/K ratio has a value of 0.59 instead of the normal figure of 1.45.

On the third day, there was still an abnormal excretion of sodium and potassium, but the difference was that sodium was still more strongly retained; but because potassium excretion was also reduced the Na/K ratio remained the same as on the first day after irradiation. During this period there was no appreciable change in the diuresis.

* We would like to thank Prof. G. N. Kassil' and Yu. K. Smirnov for offering us facilities for flame photometry in the AN SSSR laboratory (Director, Prof. N. I. Grashchenkov).

TABLE 1. Excretion of Sodium and Potassium into the Urine of Rats Before and After Irradiation

Date of determination	Number of rats	Amount of electrolytes in urine, per day in meq		
		sodium	potassium	Na/K ratio
Before irradiation (control)	108	0,58±0,07	0,4±0,025	1,45
1st day after irradiation	48	0,47±0,04	0,8±0,023	0,59
3rd " " "	42	0,33±0,045	0,55±0,03	0,60
5th " " "	33	0,64±0,11	0,32±0,05	2,0
7th " " "	24	0,56±0,10	0,52±0,056	1,07

In the following days we observed large individual variations in sodium and potassium excretion. It is worth noting that between the foruth and fifth days general symptoms of radiation sickness developed: some of the rats developed diarrhea, which by itself could cause considerable changes in electrolyte metabolism. It is therefore clear that the only results on the excretion of electrolytes which are valid are those which were obtained during the first three days after irradiation. In our opinion, the retention of sodium in the body and the increased excretion of potassium during this period are caused by an increase of mineral corticoid adrenal function. This idea is confirmed by experiments in which deoxycorticosterone acetate (DOCA) hormone, which has a mineral corticoid function, was injected.

A total dose of 5 mg of DOCA was injected in an oil solution for three days (1 mg on the 1st day, and 1 mg twice per day on the next two days). In all the animals the urine was collected on the third day after irradiation. The results are shown in Table 2.

TABLE 2. The Effect of DOCA on the Excretion of Sodium and Potassium in the Urine in Rats Before and After Irradiation

Experimental Conditions	No. of rats	Amount of electrolytes in urine, per day (in meq.)		
		Sodium	Potassium	Na/K ratio
Nonirradiated rats	108	0.58± 0.07	0.4± 0.025	1.45
Nonirradiated rats receiving DOCA	12	0.51± 0.06	0.43± 0.01	1.18
Peach oil injected 3 days after irradiation	9	0.36± 0.03	0.43± 0.01	0.83
DOCA injected 3 days after irradiation	24	0.16± 0.04	0.47± 0.03	0.34

Injection of DOCA into healthy rats has a negligible effect on sodium excretion. When DOCA is given to irradiated rats there is a great retention of sodium. In this case the effect of the irradiation and DOCA summate so that the Na/K ratio in the urine is reduced from its normal value of 1.45 to 0.34.

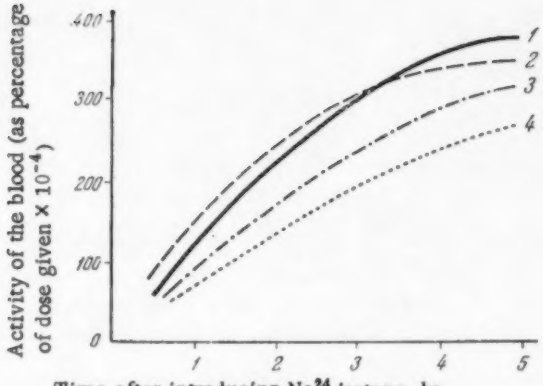
It might be expected that the marked shifts in the excretion of electrolytes with the urine would lead to corresponding changes in their amounts in the plasma. However, as can be seen from Table 3, such changes were insignificant.

The absence of any alteration in the sodium concentration in the plasma when excretion with the urine was reduced might be explained as being due to the reduced absorption of sodium from the intestine, or to an alteration in its distribution between cell and tissue fluid.

To examine the first possibility, we studied the absorption of sodium from the gastrointestinal tract, using the Na^{24} isotope. In many experiments, Na^{24} was introduced through a stomach tube and given to both control and irradiated rats; subsequently, after 15,30,60,120 minutes and later, blood was collected from the tail and its radioactivity determined. In other experiments, after the introduction of Na^{24} the body was screened with lead, and measurements of the radioactivity of the tail were made by means of an end-window counter with filter.

TABLE 3. Amounts of Sodium and Potassium in the Plasma of Control and Irradiated Rats

Date of determination	Number of rats	Amount of electrolytes, mg%	
		sodium	potassium
Before irradiation (control)	53	320	19,8
1st day after irradiation	51	320	18,5
3rd " " "	58	301	18,5



Activity of the blood in control and irradiated rats at different times after giving Na^{24}. 1) Control; 2) after 24 hours; 3) after 3 days; 4) 4 days after irradiating.

The results obtained by both methods agreed (see figure). In the first 24 hours after irradiating, the absorption of sodium remained normal, but by the third and fourth days it had been reduced. The fact that both the absorption of sodium and the plasma content remained within normal limits for the first day, while the amount excreted with the urine was reduced, appears to confirm the correctness of the view which has been reported elsewhere that both irradiation and increase of mineral adrenal corticoid function induced by other methods will lead to a liberation of potassium from cells and to its partial replacement with sodium ions from the extracellular fluid.

The following conclusions may be drawn. The effect of a minimum lethal dose of ionzing radiation applied to rats is to cause a reduced sodium and increased potassium excretion with the urine on the first and third days. During this time no appreciable changes occur in the plasma. On the third and fourth days after irradiation, less sodium is absorbed from the intestine. A total of 5 mg of DOCA given during the first three days enhances the effect of the radiation in increasing sodium excretion in the urine and leads to a marked reduction in the Na/K ratio.

These results, obtained shortly after irradiation, and before general symptoms of radiation sickness have developed, afford indirect evidence that mineral corticoid adrenal function is disturbed.

SUMMARY

Sodium and potassium urinary excretion were studied in rats irradiated with 700 r of x-rays. Na excretion was delayed and that of K increased during the first and third day after irradiation, causing a reduction in the Na/K ratio. No significant alteration in the concentration of these elements was noted in the blood plasma during the first few days after irradiation. Deoxycorticosterone acetate given to the rats after irradiation caused an even greater reduction in the Na/K ratio on the third day than did irradiation alone. Labeling with Na^{24} isotope confirmed that Na absorption from the intestine was delayed on the third and fourth days. Possibly disturbance of mineral corticoid adrenal function led to changes in mineral metabolism.

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THE INFLUENCE OF RADIOACTIVE IODINE UPON THE NONSTIMULATED AND STIMULATED THYROID GLAND

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Radioactive iodine, I^{131}, is used on an ever increasing scale, not only as a diagnostic, but also as a therapeutic preparation which permits bloodless radiostrumectomy in cases of thyrotoxicosis.

Any rational and effective clinical application of I^{131}, however, must be based on a precise and detailed analysis of the pathological changes which develop in the tissues exposed to radiation after administration of therapeutic as well as of diagnostic doses.

The doses of I^{131} used for diagnostic purposes have to a great extent been established empirically. In view of the possibility that the ionizing radiation produced by the breakdown of the I^{131} atoms accumulated in the thyroid gland exerts a toxic effect, the question arises whether exposure of the thyroid cells to this dose is indifferent and consequently safe.

There are reports in the literature according to which indicator doses of I^{131} of about 1-25 μC change the functional state of the thyroid parenchyma, producing a state of excitation and causing a number of structural changes in the cytoplasm and karyoplasm of the thyroid cell [11,15].

In our previous investigations [8,9] we were able to establish that an indicator dose of 1 μC I^{131} causes appreciable changes in the state of the biological colloids in the thyroid parenchyma of white rats, a fact which becomes above all manifest in changes in the isoelectric point and also in changes in the adsorption capacity in the first four days after the injection of the isotope into the animal body. The changes which take place in the biological colloids of the thyroid parenchyma under the influence of internal ionizing radiation arrive in waves as established in the studies of I. A. Pigalev [10] and P. D. Gorizontov [3,4]: the first wave of changes can be observed in the period between six and 48 hours and the second wave of changes in the period between 96 and 144 hours after a single injection of the isotope in question. These findings concern the thyroid gland of animals in a state of relative functional rest. In clinical practice, however, I^{131} is as a rule applied in cases of thyroid diseases which are accompanied by an increased functional activity. In this context it must be emphasized that L. A. Kashchenko [7] established in 1955 that the destructive effect of radiation becomes much more manifest if the thyroid gland is in a state of increased functional activity. On the other hand, I^{131} itself causes, as we said above, a certain functional stimulation of the thyroid parenchyma.

It was the aim of the present paper to study those changes in the biological colloids of the thyroid cells which develop as a result of exposure of the stimulated thyroid gland to the action of ionizing radiation produced by injection of an indicator dose of I^{131}.

METHOD

The experiments were carried out on two groups of male white rats weighing 180-200 g. The increased functional activity of the thyroid gland was induced by ingestion of 6-methylthiouracil [1,2,5,6]. One group of animals received the latter preparation for 25 days, after which they were given a single intraperitoneal injection of μC I^{131}. Then the injections of 6-methylthiouracil were discontinued and the changes in the colloidal system

of the thyroid parenchyma were investigated in the period of restitution after the influence of the thyrostatic agent had been discontinued.

Another group of animals were given 6-methylthiouracil with the food for 20 days; then the animals received a single injection of 1 μC I^{131}. In this group the administration of the thyrostatic agent was continued and consequently our investigation revealed the changes in the colloid system of the thyroid parenchyma which take place under the influence of internal ionizing radiation emitted by 1 μC I^{131} in both the stimulated and nonstimulated thyroid gland respectively. The animals in both groups were sacrificed 3,6,9,12,24,48,96,144 and 192 hours after the injection of I^{131}. The intensity of the adsorption process in the thyroid parenchyma was established by the method of D. N. Nasonov and V. Ya. Aleksandrov; the isoelectric point of the biological colloids in the follicular cells was established by staining reactions in buffer solution of different pH according to the method described by A. Pishinger and G. Roskin.

TABLE 1. Influence of 1 μC I^{131} on the Changes in the Biological Colloids of the Thyroid Parenchyma during the Period of Restitution after Administration of 6-Methylthiouracil Continued for 25 Days

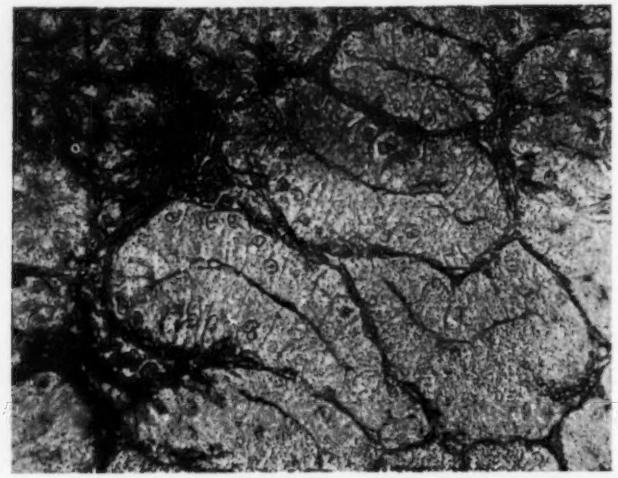
Time of experiments, hr	Average wt. of gland, mg		Intensity of neutral red adsorption, %	I^{131} uptake, %	Av. follicular cell ht., μ	Isoelectric point (pH)	
	wet wt.	dry wt.				follicular cell cytoplasm	follicular cell karyoplasm
Control animals	13,52	3,20	100,0	—	7,4	3,60	3,50
During 25-day feeding of 6-methylthiouracil	34,80	7,24	51,2	—	15,76	3,10	2,90
3 hours after I^{131} injection	35,20	8,00	59,2	15,68	13,38	3,26	3,20
6 " " " "	36,40	7,50	81,5	16,37	15,61	3,10	3,00
9 " " " "	36,00	5,83	97,0	18,81	18,40	3,37	3,20
12 " " " "	37,90	11,63	60,5	21,79	12,42	3,73	3,69
24 " " " "	41,60	11,22	56,5	9,85	14,36	3,93	3,82
48 " " " "	36,20	9,25	52,2	1,90	13,64	4,03	3,82
96 " " " "	40,00	8,90	54,4	0,95	12,66	3,93	3,82
144 " " " "	32,10	8,34	46,7	0,70	10,32	3,93	3,77
192 " " " "	31,00	8,00	49,7	0,20	9,44	3,72	3,62

TABLE 2. Influence of 1 μC I^{131} on the Changes in the Biological Colloids of the Thyroid Parenchyma in a State of Excitation Caused by Continued Administration of 6-Methylthiouracil

Time of experiments, hr	Av. wt. of gland, mg		Intensity of neutral red adsorption, %	Av. follicular cell ht. μ	Isoelectric point (pH)	
	wet wt.	dry wt.			follicular cell cytoplasm	follicular cell karyoplasm
Control	8,95	2,63	100,0	6,47	3,55	3,45
During the 20-day feeding of 6-methylthiouracil	45,20	10,52	70,0	16,58	3,20	3,10
3 hours after I^{131} injection	39,90	8,28	90,3	17,29	3,20	3,10
6 " " " "	51,80	11,27	81,0	17,62	3,20	3,10
9 " " " "	36,20	4,89	133,2	17,61	3,10	2,86
12 " " " "	33,40	6,67	130,0	17,33	3,10	2,86
24 " " " "	42,60	8,94	75,2	16,58	3,84	3,66
48 " " " "	40,90	9,63	59,3	19,20	3,84	3,66
96 " " " "	41,10	7,62	110,0	17 41	3,84	3,75
144 " " " "	23,70	6,71	89,0	15,80	—	—
192 " " " "	43,90	7,71	84,9	14,45	3,93	3,84

RESULTS

The results of the two series of experiments, given in Tables 1 and 2, I^{131} show that the internal ionizing radiation produced by an indicator dose of I^{131} intensifies the processes of stimulation of the thyroid parenchyma caused by 6-methylthiouracil to considerable degree. These swellings of the thyroid cells become more marked, a fact which is reflected in an increase of the average relative wet weight of the thyroid gland and the average height of the cells of the thyroid epithelium, as well as in the increased intensity of adsorption of neutral red compared with the adsorption found after 20-25 days' exposure to the action of thyrostatic substances, and finally in the stronger shift of the isoelectric pooint in the karyoplasm and cytoplasm of the follicular cells toward the acid side. The changes in the colloid systems of the thyroid cells enumerated above are much more marked if the exposure of the thyroid parenchyma to 6-methylthiouracil is continued (see Table 2).



Follicles in the central part of the thyroid gland of a rat treated with 6-methylthiouracil 48 hours after injection of 1 μC I^{131}. Azan stain; photomicrograph; magnification 400 ×.

Study of the structural changes in the thyroid gland of the experimental animals revealed that exposure of both the stimulated and the nonstimulated thyroid gland to internal ionizing radiation in a dose of 1 μC I^{131} leads to degenerative changes in the thyroid epithelium, changes which become manifest in the marked vacuolization of the cytoplasm, polymorphism of the nuclei, karyorrhexis and pycnosis(see figure). If the administration of 6-methylthiouracil is continued, the degenerative changes in the thyroid gland are much more marked.

SUMMARY

Administration of a single 1 μC I^{131} dose to albino male rats provokes considerable changes in their thyroid glands and marked alterations in the physico-chemical composition of the cytoplasm and karyoplasm of the thyroid epithelium. In the first 2 days this is manifested in the dislocation of the isoelectric point of the above cellular components toward increased acidity, in the intensified neutral red sorption, in the increased mean height of follicular cells and the mean relative crude weight of the gland.

The aforementioned changes proved more pronounced in the thyroid gland previously activated by the action of 6-methylthiouracil. With the continuing 6-methylthiouracil action on the thyroid gland after radioiodine injection, the thyroid parenchyma becomes a scene of the following pathological changes: marked polymorphism of the follicular epithelium cells, large perinuclear vacuolization, a rise in the number of nuclear pycnoses and karyorrhexis and, here and there, desquamation into the follicular cavity. Thus, radioiodine affects both the unstimulated and stimulated thyroid; in the latter case the effect is considerably intensified even by small doses of radioiodine.

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METASTASIS FORMATION OF BROWN-PEARCE TUMOR INOCULATED INTO DIFFERENT PARTS OF THE STOMACH UNDER NORMAL CONDITIONS AND AFTER EXPOSURE OF THE LIVER TO CARBON TETRACHLORIDE

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The liver is the organ which in cases of stomach cancer is most frequently affected by metastases. According to the findings of L. M. Nisnevich [10], metastases of stomach cancer in the liver are found in 28.3% of cases, and according to the data of F. I. Pozhariskii [15], in 36.4% of cases, a fact which puts the liver into the first place among those organs in which metastases of stomach cancer develop. This fact is also reported in the papers published by a number of other authors [2,12]. Cancer of the cardia part of the stomach is particularly dangerous, as it is difficult to recognize, a fact which prevents the early diagnosis [5,16].

Numerous clinicians and morbid anatomists [2,3,5,10,13] believe this form of cancer to be particularly malignant as far as the formation of metastases is concerned. There are also reports [4] according to which the lymphatic system of the cardia part of the stomach is connected with the efferent lymph vessels of the liver.

In view of the facts discussed above, study of the conditions enchancing the growth of metastases originating from a stomach carcinoma in the liver seems to be of particular importance.

There are reports in the literature according to which the development of precancerous conditions induced by the administration of carcinogenic substances, as well as the growth of the tumor in the liver, depends on the functional state of that organ [17,14].

N. P. Kochneva [8] transplanted Brown-Pearce tumors into the stomach wall of rabbits and observed the appearance of liver metastases after not less than two months. This author does not state the site of inoculation and the dose of tumor emulsion employed.

Fig. 1. The wall of the cardia part of the stomach of a rabbit to the mucosa site with a Brown-Pearce tumor on the 24th day after the inoculation.

In the present paper we set ourselves the task to study the metastasis formation of Brown-Pearce tumors transplanted into the stomach wall of rabbits in relation to the localization of the tumor in the stomach and to functional changes in the liver evoked by administration of a hepatotoxic substance.

METHOD

The experiments were carried out on male rabbits weighing 2500-2900 g. The Brown-Pearce tumor was inoculated on to the serous membrane of the stomach by injection of 0.4 ml of a 10% tumor emulsion. In the first series of experiments we used 16 rabbits which were distributed into four groups, each consisting of four animals. In the first group the tumor was inoculated into the pylorus, in the second group into the cardia, in the third group into the greater curvature and in the fourth group into the fundus.

In the second series of experiments the experimental animals (17 rabbits) were given for three subsequent days

carbon tetrachloride in a dose of 0.25 ml per kg weight by subcutaneous injection into the back. On the third day after the beginning of the experiments the tumor was inoculated into the cardia of all experimental and all 15 control rabbits. On the 13th day after the implantation of the tumor the experimental animals were again given an injection of carbon tetrachloride in the same dose.

The animals were sacrificed by air embolism on the 21st-23rd day after the implantation of the tumor. Autopsy revealed at the site of inoculation a tumor of circular shape, of 0.5 to 2-2.5 cm diameter, which infiltrated the gastric mucosa and formed on the mucosa surface ulcers with elevated rounded edges (Fig. 1). In some rabbits metastases were found in the stomach wall; more frequently remote metastases occurred on the peritoneum, the mesentery, in the intestine, omentum, kidneys, liver and lungs. The metastasis formation in the organs was studied according to an arbitrarily chosen fixed scheme, taking into account the number and size of the metastases expressed numerically. Metastases of 0.2 cm size were given one mark, metastases of 0.2-0.5 cm size were given two marks, and metastases over 0.5 cm three marks. These numbers were correspondingly increased depending on the number of metastases: up to ten metastases one more mark was given; between 10 and 30 metastases two more marks; if the number of metastases exceeded 30, three more marks were given. The numerical indices thus obtained were evaluated statistically: the coefficient of coincidence (X^2) and the probability P were calculated with the aid of Fisher's tables [20].

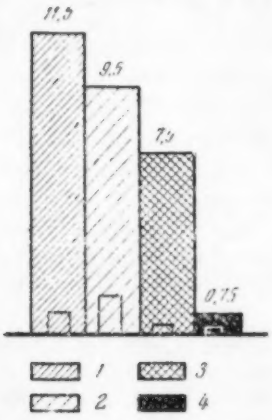

Fig. 2. The different degree of metastasis formation by a Brown-Pearce tumor in the organs of rabbits after inoculation of the tumor into various parts of the stomach. 1) Inoculations into the pylorus; 2) into the cardia; 3) into the region of the greater curvature; 4) into the fundus. The large columns illustrate metastasis formation in the internal organs, the smaller columns metastasis formation in the liver.

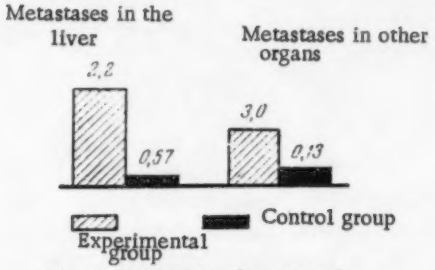

Fig. 3. Metastasis formation by Brown-Pearce tumor in the liver and other internal organs of rabbits after inoculation of the tumor into the cardia and injection of carbon tetrachloride.

RESULTS

The results of the first series of experiments are set forth in Fig. 2.

Comparison of the findings concerning metastasis formation in the four groups of rabbits revealed that inoculation of the tumor into the pylorus and the cardia of the stomach were followed by the most intensive metastasis formation and inoculation into the fundus produced least of all metastases; results which more or less coincide with clinical observations [2].

The metastases in the liver were found after inoculation into all four localizations used by us, but the most intensive metastasis formation in the liver could be observed after inoculation of the tumor into the cardia.

The results obtained in the second series of experiments are set forth in Fig. 3. If one compares the metastasis formation in the experimental and control group, respectively, it appears that in those rabbits which had been given injections of carbon tetrachloride the metastasis formation was somewhat more intensive in the internal organs, but the number of metastases in the liver showed a particularly marked increase. The results of these experiments concerning the metastasis formation in the liver are statistically highly significant: the probability $P < 0.01$, with a coefficient of coincidence $X^2=14.98$. The findings concerning metastasis formation in other internal organs are less significant: P varies between 0.5 and 0.3, with a coefficient of coincidence $X^2=5.15$.

Numerous experimental data obtained in the laboratory of A. D. Speranskii and R. E. Kavetskii [1,6,7,9,18,19] have shown the importance of reflex influences in the process of metastasis formation, which influences may originate from the primary focus of tumorous growth as well as from various other parts of the body. These findings underline the importance of trophic changes in the tissues and organs in the metastasis formation, as these changes create conditions which may enhance or inhibit the tumorous growth.

Our experimental findings show that dystrophic disorders in the liver which develop under the influence of carbon tetrachloride create conditions favorable for the growth of metastases of stomach tumors in that organ, a fact which may be of importance in studies concerning the pathogenesis of metastasis formation in cases of stomach cancer.

SUMMARY

The author studied metastasization of Brown-Pearce tumor (inoculated into different portions of the rabbit stomach wall) into the liver and other internal organs. Observations were also made during the administration of carbon tetrachloride (hepatotoxin). The most extensive metastasization into the liver was observed when the tumour was inoculated into the cardia portion of the stomach. Hepatic functional disturbance intensified the metastasization of gastric tumours into the liver.

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THE GROWTH OF A PRIMARY TUMOR (SARCOMA M-1) DURING THE TRAINING OF RATS TO HYPOXIA IN THE LOW PRESSURE CHAMBER

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The detection of conditions which enhance the resistance of the animal body to malignant growths and study of the mechanism governing the defensive and compensatory reactions which take place during that process are undoubtedly of great importance for the solution of problems connected with the pathogenesis of tumors.

In addition to immunological investigations, to which in recent years considerable attention has been paid, investigations should also be continued with regard to the influence exerted by unspecific resistance upon the tumorous process.

It is well known that exposure of the animal body to certain stimuli leads to an increased resistance, not only to the stimulus in question, but also to other factors.

For example, animals adapted to the stimulus of cold (hypothermia) proved to be resistant, not only to cold, but also to oxygen deficiency due to various causes [5,6].

Animals which had been trained to conditions of low barometric pressure acquired an increased resistance to the effects of rarefied atmosphere as well as to hypothermia [4].

Training to hypoxia, however, sometimes produces resistance of the animal body in what seems to be quite unexpected directions: e.g., training of animals to hypoxia increased their resistance to irradiation with x-rays [1].

From this point of view studies concerning the influence of training to hypoxia upon the tumorous growth seem to be of certain interest. This question arose also on the basis of certain preliminary results obtained in our laboratory by V. N. Popov.

It was the aim of the present experiments to study the influence of adaptation of rats to hypoxia in conditions of low barometric pressure upon the growth of primary rat tumors (M-1 sarcoma).

METHOD

In our experiments the primary tumor was implanted under the skin of the left thigh (inoculation of 0.2 ml of a 25% tumor tissue suspension in normal saline). The training in the low pressure chamber was performed by keeping the rats daily in the chamber. The training was begun simultaneously with the implantation of the tumor and continued for 10-14 days. The "height" changed depending on the experimental conditions between 6000 m over sea level (353 mm Hg: "soft" training) and 10,750 m over sea level (175 mm Hg: "hard" training). The exposure of the rats to the "height" in question was continued for 50-60 min.

RESULTS

Our findings concerning the relation between the tumorous growth and the training conditions are summed up in the table.

The Influence of Training Conditions in the Low Pressure Chamber upon the Growth of a Primary Tumor (M-1 Sarcoma)

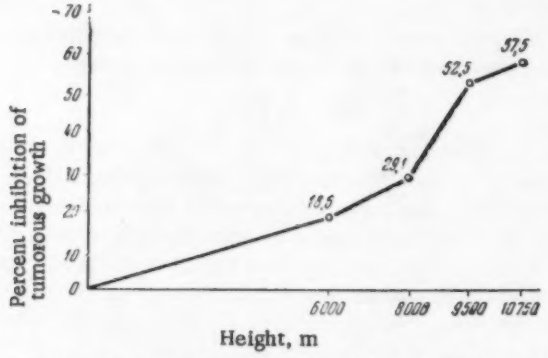
Expt. No.	"Height," m								
	8000–10750			6000			control rats (not trained)		
	no. of rats	average tumor weight, g	no. of rats failing to develop tumor	no. of rats	average tumor weight, g	no. of rats failing to develop tumor	no. of rats	average tumor weight, g	no. of rats failing to develop tumor
1	20	5,11	2	20	6,59	0	10	9,3	0
2	22	3,65	2	22	6,32	1	22	7,7	0
3	15	4,37	0	15	8,99	1	15	10,3	0
Summarized data	57	$M_1 \pm m_1 = 4,37 \pm 0,58$*	4	57	$M_2 \pm m_2 = 7,3 \pm 0,73$†	47	$M_3 \pm m_3 = 9,1 \pm 0,22$		

*Significance of the difference $M_3 - M_1$: $t = 9.8$, $P = 0.001$.
†Significance of the difference $M_3 - M_2$: $t = 2.6$, $P = 0.01$.

The table shows that training of rats in the low pressure chamber inhibits the growth of the tumor and that the "hard" training 8000-10,750 m "over sea level" causes a more marked inhibition. Thus, in the control rats the average weight of the tumor was equal to 9.1 g in all three experiments, but in the rats exposed to " hard " training the average weight of the tumor was only 4.37 g, i. e., less than half as much. The average weight of the tumor in rats exposed to "soft" training (6000 m) was equal to 7.3 g.

The cases in which the tumor failed to develop are of certain interest for the evaluation of the effect exerted by the training of rats in the low pressure chamber upon the growth of the tumor. Among 57 rats trained under "hard" conditions of hypoxia the tumor failed to develop in four rats, and among a similar number of rats trained under "soft" conditions of hypoxia the tumor failed to develop in two cases, whereas in the control group the tumor developed in all 57 rats.

Depending on the experimental conditions, the "height" at which the training was performed was different for various groups of experimental animals and was equal to 6000, 8000, 9500 and 10,750 m, respectively. If we investigate the findings concerning the growth of the tumor in the same order, a not less convincing picture of inhibition of the tumorous growth depending on the "height" of training can be obtained (see figure).



Relation between the inhibition of the tumorous growth and the "height" of rise in the low pressure chamber.

The percent of inhibition of the tumorous growth was calculated by the formula:

$$\frac{C_c - C_e}{C_c} \times 100,$$

in which C_c represents the average weight in the control group and C_e the average weight of the tumor in the experimental group.

The figure shows that the percent of inhibition of the tumorous growth increased parallel to the "height" of training.

The development of the mechanisms which inhibit the tumorous growth has proved to be directly related to the "height" at which the training to hypoxia was performed.

The adaptation to hypoxia is a very complex process. This process involves a number of compensatory mechanisms: changes in respiration and circulation, mobilization of carbohydrate stores, adaptative changes in the metabolism, the number of working capillaries in the brain increases, and so on. As the adaptation advances those compensatory mechanisms which are aimed at an improved utilization of oxygen by the tissues acquire predominant importance [7].

A certain group within the above (far from complete) enumeration of the mechanisms which secure the adaptation of the animal body to lack of oxygen, which adaptation develops during the training to hypoxia, exerts an inhibitory effect upon the growth of the primary tumor.

Studies of the biological nature of these mechanisms, which apparently belong to the mechanisms of unspecific resistance, seems to be of great interest for the clinical oncologist. The search for adequate methods of acting upon an individual affected by a malignant tumor with the aim of stimulating these mechanisms and consequently of developing the unspecific resistance is definitely important.

Recent studies on the influence exerted by repeated exposure of animals to conditions of hypoxia were met with considerable interest. In 1959 an article devoted to that problem was published by G. G. Korobkov [2]. This work is of certain interest, but regrettably the author failed to mention the number of experimental animals and the significance of the results obtained; it is consequently difficult to judge his conclusions.

The present paper thus briefly reports the fact that training of rats in low pressure chambers exerts an inhibitory influence upon tumorous growth. Our findings warrant the conclusion that the inhibitory effect upon the tumorous growth is the more marked, the harder the conditions of training in the low pressure chamber. The development of the mechanisms which inhibit the growth of M-1 sarcoma directly depends on the degree of training to hypoxia to which the rats are subjected.

SUMMARY

The effect of hypoxic training of rats on the growth of transplantable tumors (sarcoma M-1) was studied in experiments involving 161 animals. In training the rats at an "altitude" of 6,000 m, the tumor growth was delayed by 18.6 per cent. With the rise of the training "altitude" there is a progressively increasing development of the tumor growth-inhibiting mechanisms. At the maximal "altitude" of training (10,750 m) the tumor growth inhibition reached 57.5%.

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MITOTIC ACTIVITY DURING MUSCULAR EXERTION

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The functional activity of the cells and the general functional activity of the body represent one of the factors which determine the daily pattern of cell division in the animal body [1,6,7]. The interrelation between cell division and the functional activity has attracted the attention of research workers for a long time. Changes in the mitotic activity connected with the general functional exertion of the whole body, however, have hardly been studied. Bullough [7] has shown that during intensive muscular exertion (running in a rotating drum) the mitotic activity decreases. And conversely: under conditions of drug-induced sleep the multiplication of cells becomes more intensive. The cause of these changes remains obscure.

In the present paper we studied changes in the mitotic activity during short-lasting muscular exertion and made attempts to establish the mechanism of these changes.

METHOD

The experiments were carried out on frogs and on three-month-old male white mice. The mitotic activity in the mice was assessed in the corneal epithelium. The intensity of the mitotic activity was assessed by the number of cells in a state of division within a standard area (1.65 mm^2), by the percentage proportion of various mitotic phases and by the phase coefficient. The findings were evaluated statistically by the method of Fisher and Student.

In the first series of experiments we studied changes in the mitotic activity during intensive muscular exertion. The intensive exertion of the mice consisted of swimming in a water bath at a temperature of 37°C. The swimming was continued for 45 min. During the week preceding the experiment the mice were subjected to preliminary daily training (five min). At various times after the swimming for 45 min, the mice were killed (after 45 min, one hour, 2 ½ hours, four hours and six hours after the beginning of the exertion). In all experiments the mice were killed at the same time of the day. Animals kept under similar conditions but not subjected to muscular exertion served as control.

In the second series similar experiments were carried out on white mice after preliminary bilateral adrenalectomy. The bilateral adrenalectomy was carried out three-four days before the experiments.

In the third series of experiments (carried out jointly with Yu. B. Temper) we studied the influence exerted by the metabolic products of working muscles upon the cleavage of frogs' eggs. The hind legs of frogs were perfused with Ringer's solution through the aorta by the method of Trendelenburg. The perfusion was repeated several times with one and the same solution (1-1.5 ml). The perfusate was collected before (control) and after (experimental) stimulation of the muscles. The contraction of the muscles in various groups of experimental animals was induced either by direct stimulation or by stimulation of the nerves in the lumbosacral plexus. The stimulation was induced by induction current through an interrupter (30-40 stimuli per min). The contraction of the muscles was continued for 3-5 min and followed by a rest period. The periods of work and rest were repeated 3-4 times until the muscle became tired. Eggs of frogs (Rana nigromaculata, R. chensinensis) were submerged into the perfusate. The times of appearance and of completion of consecutive cleavage furrows were recorded under the microscope.

In the fourth series of experiments we studied the changes in the mitotic activity of corneal epithelium of mice after injection of perfusate from working muscles. The perfusate was administered by intraperitoneal injection (0.2-0.3 ml). The material was investigated 1-1 ½ hours after the injection.

RESULTS

The results of the experiments showed that during intensive muscular exertion the mitotic activity decreases in the corneal epithelium (Table 1).

TABLE 1. Changes in the Mitotic Activity in the Corneal Epithelium of Mice during Muscular Exertion

Group of mice	No. of animals	Time elapsed after beginning of experiment	No. of mitoses	Phase proportion, %				P
				prophase	metaphase	anaphase	telophase	
Control	5	—	134	18,4	30	6,6	45	0,001
Experimental	8	1 hr 10 min	38	11	18	4	67	
Control	6		86	12	33	8	47	
Experimental	6	2½ hr	21	60	8	14	18	0,001
Control	5	—	96	35	19	4	42	
Experimental	6	45 min	67	27	47	5	21	0,04
"	5	4 hr	125	25	18	6	51	0,08
Control	5	—	124	25	14	8	53	
Experimental	5	6 hr	104	36	14	7	43	0,1

Already within 45 min after the beginning of the exertion (i.e., immediately after completion of the exertion), a decrease in the mitotic activity could be observed in some animals. After one hour the number of cells in a state of division decreased almost to one quarter, and 2½ hours after the beginning of the exertion the mitotic activity was still on a very low level. Four hours after the exertion the mitotic activity was restored and even exceeded slightly the original level. Six hours after the beginning of the exertion the intensity of cell multiplication had reached its normal values. The decrease in the mitotic activity was accompanied by a gradual fall in the number of early mitotic phases. A new wave of mitoses appeared already within 2½ hours, when the level of mitotic activity was still very low (increase in the relative proportion of prophases). The shifts in the percentage proportion of the various mitotic phases warrant the assumption that the changes in the mitotic activity during muscular exertion are due to an inhibition preventing the cells from starting the process of division.

The character of changes in the mitotic activity during muscular exertion suggests that these changes are connected with an increased production of adrenaline. Adrenaline represents a powerful factor which prevents the cells from beginning the process of division. On the other hand, intensive muscular exertion is followed by a hypersecretion of adrenaline [8]. Taking into account these facts, we carried out the second series of experiments, in which we studied the changes in the mitotic activity during muscular exertion in mice subjected to bilateral adrenalectomy.

TABLE 2. Changes in the Mitotic Activity in the Corneal Epithelium of Adrenalectomized Mice during Muscular Exertion

Group of mice	No. of animals	Time elapsed after beginning of expt.	No. of mitoses	Phase proportion, %				P
				prophase	metaphase	anaphase	telophase	
Control	5	—	96	23	38	6	33	
Experimental	6	1 hr 10 min	118	5	26	5	64	0,1
Control	6	—	110	23	32	4	41	
Experimental	6	2 hr	11	38	0	0	62	0,001

The results of these experiments showed (Table 2) that one hour ten min after the beginning of the exertion no decrease in the mitotic activity occurred in the adrenalectomized animals. Only a relative fall in the number of prophases could be observed, a phenomenon usually observed in the beginning stages of the mitotic process. A marked inhibition of cell division could be observed in the adrenalectomized mice only after two hours.

Comparison of the mitotic activity in healthy and adrenalectomized mice, respectively, shows (Fig. 1) that in the absence of the adrenal glands the decrease in the number of cell divisions during muscular exertion is delayed.

In nonoperated mice the mitotic activity begins to decrease within 45 min and after one hour is already on a very low level. In adrenalectomized animals this decrease is marked only two hours after the beginning of the exertion. The initial inhibition of the mitotic activity during intensive exertion is apparently due to the secretion of adrenaline into the blood stream. The subsequent inhibition of mitosis is apparently induced by other factors. The findings of Heilbrunn and Wilson [10] warrant the assumption that these factors are the metabolic products of the working (tired) muscle. The above authors observed that sea water in which the working muscle had been kept inhibits cell division.

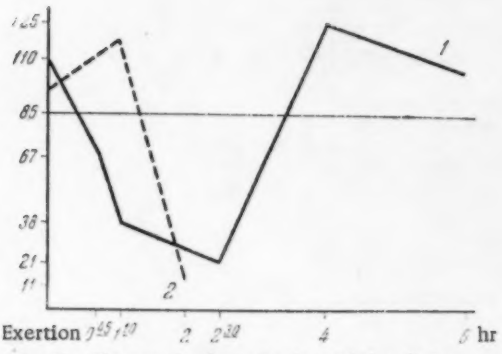

Fig. 1. Changes in the mitotic activity during muscular exertion in nonoperated (1) and adrenalectomized (2) mice. On the abscissa: the time (in hours); on the ordinate the number of mitoses were plotted.

In the third series of experiments we studied the rate of cleavage of frogs'eggs sumerged into the perfusion liquid which had been passing through the blood vessels in the hind legs of frogs. The control eggs were kept in a perfusate collected before the muscular contraction. The eggs of the experimental group were submerged into a perfusate collected after the muscular exertion.

The results of these experiments showed that certain substances develop in the working muscles which inhibit the cleavage of the egg cells (Fig. 2). The inhibition of the cleavage took place soon after submersion of the eggs into the perfusate and persisted throughout the 3-4 hours of observation.

In the majority of experiments the effect was more marked in the beginning of the observation and became weaker toward the end of the experiment. The development of six-seven cleavage furrows in the experimental eggs was on the whole delayed for 30-35 min compared

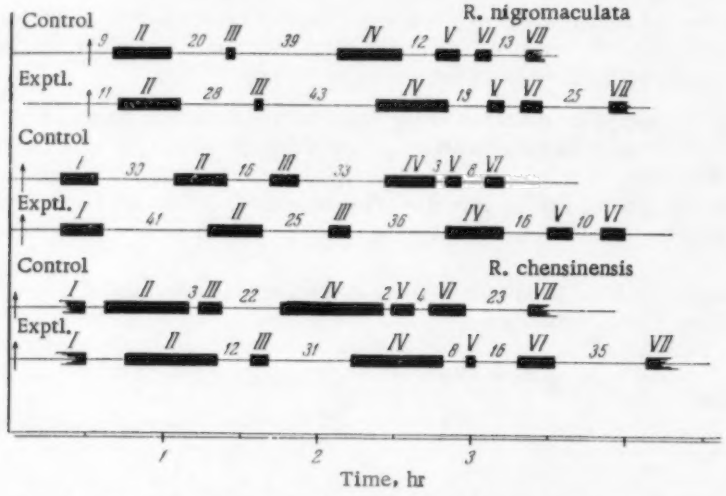

Fig. 2. The rate of cleavage of frogs'eggs. The thick parts of the lines illustrate the time of cleavage, the thin parts the stage of interkinesis. The Roman numbers indicate the consecutive cleavage furrows; the Arabic numbers show the duration of the interkinesis.

with the control eggs. The delay in the cleavage of the eggs was mainly due to a prolonged stage of interkinesis. The speed of division itself was on the whole unaffected and the deviations which could be observed in some cases did not exceed the range of possible error. The duration of the interkinesis was appreciably prolonged (in some cases by 10-12 min).

The effect of the metabolic products developing in the working muscles is of unspecific character. The perfusion liquid which had been passed through the blood vessels of frog muscles before and after contraction was injected into white mice of the control and the experimental group respectively. Injection of the perfusate from working muscles caused a decrease in the mitotic activity in the corneal epithelium (Table 3). This decrease was relatively small and not always sufficiently marked, but it occurred in all three groups of experiments performed by us. In two groups of similar experiments a decrease in the relative proportion of prophases could be observed.

TABLE 3. The Influence of Perfusate Which Had Been Passed through Working Muscles upon the Mitotic Activity in the Corneal Epithelium of Mice

Group of animals	No. of animals	No. of mitoses	Phase proportion, %				P
			pro-phase	meta-phase	ana-phase	telo-phase	
Control	5	136	24	38	4	34	<0,001
Experimental	5	82	18	38	5	39	
Control	5	176	26,5	31	4,5	38	
Experimental	5	101	17	38	4	40	0,01
Control	6	80	25	29	6	39	
Experimental	6	55	24	37	4	40	0,02

The results of our experiments thus warrant the assumption that the second factor responsible for the inhibition of cell division during intensive muscular exertion consists of the metabolic products developing in the working (exhausted) muscle. We were unable to establish the nature of these products. Biochemical investigations had shown that in the exhausted muscle a breakdown of nucleotides and formation of free purine bases place [3]. Taking into account the fact that nitrogen bases suppress the mitotic activity [2,5,9], we made attempts to abolish the inhibition of mitosis during muscular exertion by preliminary injection of sodium citrate. This preparation inhibits the breakdown of nucleotides and ATP in the muscles [4]. The laws of these experiments were not sufficiently consistent, and we were unable to come to definite conclusions.

The results of the experiments described above thus show that during short-lasting intensive muscular exertion the mitotic activity undergoes a marked decrease. These changes are caused by at least two factors: the initial decrease in the mitotic activity is apparently caused by the increased secretion of adrenaline, and the subsequent inhibition of cell division is caused by metabolic products liberated from the working (exhausted) muscle. Changes in the mitotic activity found during short-lasting muscular exertion enable us to understand certain features characterizing the pattern of mitoses in the animal body. For a complete analysis of the daily rhythm of cell division from all aspects, however, further studies concerning the changes in the mitotic activity during prolonged but moderate functional exertion are necessary. It is quite possible that under the latter conditions the cell divisions will be affected in another way than during short-lasting intensive exertion.

SUMMARY

Brief muscular exertions in mice lead to a marked reduction of mitotic activity in the corneal epithelium associated with the delay in the onset of mitosis. In adrenalectomized animals cellular mitosis is inhibited later than in control animals. The perfusion fluid passing through the vessels of the strained muscle inhibits the cleavage of frog eggs. The deceleration of the cleavage rate is connected mainly with the prolongation of interkinesis. The action of metabolites of the working muscle is nonspecific. At least two factors are involved in the reduction of mitotic activity during physical exertion: initial reduction of mitotic activity is caused by intensified adrenalin secretion, whereas subsequent inhibition of mitoses is induced by the metabolites of the working muscle.

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CHANGES IN THE MITOTIC ACTIVITY OF THE RENAL EPITHELIUM IN RELATION TO THE FUNCTIONAL ACTIVITY OF THE KIDNEY

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The changes in the interrelation between the functional activity of cells and their division has attracted the attention of cytologists for a long time. This is no coincidence, as the functional activity of cells is one of the factors which determine the pattern of cell division in the animal body [1,3]. The data concerning the relation between functional activity of cells and their division are of different value. To elucidate this question the majority of authors used pharmacological methods of changing the functional activity of organs (pilocarpine, adrenaline), or the method of injecting supravital stains [2,5,6,7]. The results of these investigations must be assessed with great caution, as they do not exclude a possible direct influence of the pharmacological preparations and stains used upon the cell multiplication independently of the functional activity of the organ in question. In view of this fact, physiological methods of stimulating cellular activity or the detection of relations between the natural function of cells and their division offer better prospects [1,3,4]. Blumenfeld [3] found, using this method, a reverse proportion between the functional activity of the organs and their mitotic activity. I. A. Alov [1] had shown that distortion of the normal order of functional activity in the digestive organs leads to corresponding changes in the daily periodicity of mitotic activity in the lungs and in the gut.

In the present study we made attempts to establish a relation between the functional activity of the kidneys and the cell division in the renal epithelium, using both physiological and pharmacological methods of stimulating the activity of that organ.

METHOD

The experiments were carried out on white mice (males aged 2-2 $\frac{1}{2}$ months) and rats (males aged 3-5 months). The mitotic activity was assessed in kidney sections (10 μ) stained with hematoxylin-eosin. The cells in a state of division were counted in the epithelium of the chief part of the nephron in four sections. The intensity of the mitotic activity was assessed by the number of cells in a state of division found within a standard area (3.08 mm^2), by the percentage proportion of the various mitotic phases and by the phase coefficient. The findings obtained were evaluated statistically by the method of Fisher and Student.

Three series of experiments were carried out on 157 animals. In the first series of experiments we studied the relation between the daily pattern of renal function in rats and the mitotic activity found in that organ. The changes in the functional activity of the kidney in the course of the day were assessed by the rate of diuresis. The animals were kept in cages with a funnel-shaped bottom. Every three hours the quantity of urine passed by the animals was recorded. The daily rhythm of diuresis was observed for eight days. After this period the animals were killed at various times corresponding to the maximum and minimum daily activity of the kidneys.

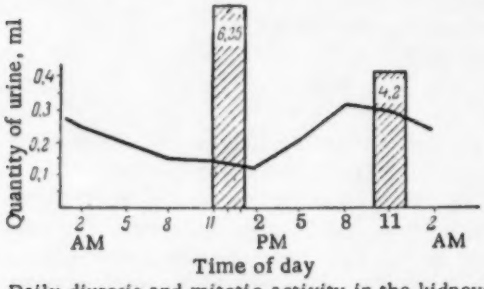
In the second series of experiments we studied the changes in the mitotic activity in the renal epithelium while the animal body was flooded with water. Normal saline was administered by subcutaneous injection. Mice (of 15-20 g weight) were given 1 ml and rats (of 80-100 g weight) 5 ml normal saline. Animals which were given only a prick with the needle without injection of normal saline served as control. The mitotic activity in the kidneys was assessed 1,2 and 3 hours after the injection.

In the third series of experiments the animals were given diuretin (0.1% solution). White mice were given diuretin in a dose of 1 mg, rats in a dose of 5 mg per animal. The total quantity of liquid injected in this series

of experiments was the same as the amount of liquid used to flood the animals with water in the previous series of experiments.

RESULTS

The findings obtained after continuous observation lasting for eight days showed that the renal activity of rats is governed by a well marked daily rhythm with a minimum and maximum (see figure). The minimum diuresis takes place in daytime between 8 a.m. and 2 p.m. then follows a rapid increase in the diuresis up to a maximum observed between 8 p.m. and 11 p.m. From then on the curve of diuresis shows a slow fall gradually reaching the minimum level.



Daily diuresis and mitotic activity in the kidneys of rats. On the ordinate: the quantity of urine, on the abscissa: the time (in hours) are plotted. The columns show the mitotic activity in the kidneys during the period of maximum and minimum renal activity.

In view of these findings we investigated the mitotic activity of the animals at different times which coincided with the minimum and maximum renal activity. Twelve rats were killed at the peak of diuresis (11 p.m.) and 11 rats during the minimum excretion of urine (12 noon).

The results of these experiments showed that the mitotic activity in the kidneys as well as in other organs undergoes certain changes in the course of the day. High indices of mitotic activity were found in the morning hours and low indices at night (P=0.005). Similar to the results obtained in the experiments of Blumenfeld [3], the reverse proportion between the functional activity and the mitotic activity of that organ deserves attention. The high indices of mitotic activity coincide with the minimum activity of the kidneys, and conversely at the peak of diuresis the mitotic activity was low (see figure).

Changes in the Mitotic Activity in the Renal Epithelium of Rats and Mice after Flooding with Water

Animals	Group	No. of animals	Time after water admin., hr	No. of mitoses	Phase coefficient*	P
Rats	Control	7	—	26,1	2,8	
	Experimental	7	2	16,1	2,1	<0,01
		6	3	17,3	2,0	<0,02
Mice	Control	9	—	5,4	2,6	
	Experimental	10	1	3,4	2,8	0,03
	Control	10	—	11,1	2,3	
	Experimental	10	2	8,3	2,2	0,2
		10	3	10,0	2,7	0,3

* The coefficient reflects the relation between the number of prophases and metaphases, on the one hand, and the number of anaphases and telophases, on the other hand.

The above data warrant the assumption that the functional activity of the organ leads to a decrease in the intensity of cell multiplication. It would, however, be premature to draw a final conclusion, as a coincidence of two processes in time does not always imply a causative link between these processes. In view of this consideration we undertook the second series of experiments, in which we studied the changes in the mitotic activity of the kidneys in animals flooded with water.

The results of these experiments showed that the short-lasting increase of the diuretic function of the kidneys leads to a decrease in the mitotic activity in that organ (see table). The most striking data were obtained in experiments on rats. Two hours after injection of normal saline the mitotic activity in the chief parts of the kidneys decreased more than $1\frac{1}{2}$ times. After three hours the mitotic activity was still on a very low level.

Similar results were obtained in experiments on mice. In the kidneys of the control mice the mitotic activity usually varies within a fairly wide range and consequently the experimental results are not always sufficiently consistent. In all experiments on mice a decrease of the mitotic activity could be observed 1-2 hours after administration of water which was more or less marked in different experiments. Three hours after injection of normal saline the mitotic activity usually returned to the original level. The results of these experiments thus warrant the assumption that the coincidence between the low mitotic activity and the maximum of the diuretic function was not of incidental character. A short-lasting functional exertion of the kidneys leads to a decrease in the intensity of cell multiplication.

In the third series of experiments we studied changes in the mitotic activity of the kidneys after injection of diuretin. The preparation was administered in the same volume of normal saline as in the preceding series of experiments. Although in this group of experiments two factors increasing the activity of the kidneys were used (flooding with water + a diuretic substance) the results were inconsistent and did not enable us to draw definite conclusions. In one group of experiments the injection of diuretin slightly decreased the mitotic activity; in two other groups the mitotic activity increased. The changes were insignificant and not sufficiently consistent. We gained the impression that diuretin in itself exerts an influence upon cell division independently of its diuretic effect. In our opinion the use of pharmacological preparations for the study of the relation between the cell division and functional activity represents an unreliable method for the solution of that problem.

Comparison of the daily pattern of renal function with the intensity of cell multiplication in that organ and experiments consisting of the flooding of the animal body with water thus showed that a definite relation exists between the cell division and the functional activity of the kidneys. A reverse proportion between these processes could be established. At the peak of functional activity and during short-lasting functional exertion the cell division appeared to be delayed. This conclusion is naturally valid only for the conditions of our experiments, i. e., for short-lasting intensive exertion of the kidneys. It cannot be excluded that during prolonged but moderate functional activity other interrelations between renal function and cell division in the kidneys might be found.

SUMMARY

Rats and mice were experimented upon to inquire into changes occurring in the mitotic activity of the kidney in various functional conditions of the organ. An inverse relationship was found to exist between the functional activity of the organ and mitosis. High level of mitotic activity was noted during hours of minimal diuresis, and a low one at the time of maximal diuresis. A brief increase of the renal function achieved by way of water loading resulted in a reduction of mitotic activity in this organ.

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THE EFFECT OF CHONDROITIN SULFATE (CHONSURIDUM) ON COLLAGEN IN VIVO

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(Presented by Active Member AMN SSSR N. A. Kraevskii)
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We here report a study of the effect of acid mucopolysaccharide chondroitin sulfate on regeneration in vivo.

The theoretical basis for the clinical application of chondroitin sulfate in cases where wounds have remained unhealed for a long time was the phenomenon previously reported of disturbed relationships between components of the enzyme-substrate systems hyaluronidase-hyaluronic acid and chodroitin sulfuric acid [1]. In various pathological conditions, the reaction may be shifted either so that there is an accumulation of acid mucopolysaccharides (in cicatrization, hematoma, tissues of a false articulation, etc.) or the change may be such as to reduce the amount of these components. It has been found that in wounds which remain unhealed for a long time and which contain soft granulation tissue, the amount of mucopolysaccharides is reduced. This circumstance was the reason for developing the standard preparation of chondroitin sulfate for stimulating the repair processes in wounds which heal with difficulty.

The preparation chonsuridum† was obtained by our modification of Einbinder and Shubert's method [5], which enabled a sufficiently high degree of polymerization of the molecules and minimal loss of protein into the solution during extraction to be attained. The preparation was identified by various methods: 1) by electrolytic separation using the method of Rienits [10]; 2) by a turbidity method, using the method of Mathews and his co-workers [7]; 3) by determining the hexosamine content, by the method of Elson and Morgan as modified by Boas [4]; 4) by separating with ion exchange resins using Gardell's method [6].

We here give the hexosamine and nitrogen content of chondroitin sulfate and, for comparison, the results of several others authors who separated it under laboratory conditions using various more complex methods (see table).

When the preparation was separated using the ion exchange resin Dowex-50, it was found that of the total hexoseamines 85% was galactosamine, and 15% was glucosamine (Fig. 1).

In clinical practice, favorable results were obtained by using chondroitin sulfate in cases of operative wounds showing granulation tissue, and trophic abscesses; less good results were obtained after compound fractures, particularly when they were complicated by osteomyelitis, and in third degree burns. No adverse side effects were noted.

The general reaction of the organism to the local external application of the preparation was controlled by determining the protein fraction of the blood serum by paper electrophoresis, the glucoprotein fraction from serum mucoid using Winzler's method [11] as modified by Menini and his co-workers [8], and the sial acids using the

* Deceased.

† The preparation was made by B. S. Kasavina and G. D. Zenkevich of the Central Institute of Traumatology and Orthopedics in collaboration with M. Z. Alfer'eva and L. S. Pozhariiskaya from the Scientific Research Institute of the Meat Industry and by A. O. Pashkov, S. S. Togunov, and T. S. Bogushevich of the Leningrad Pharmaceutical Works.

Analysis of a Preparation of Chondroitin Sulfate

Source	Hexoseamine	Nitrogen	Author
	%		
Cartilage (tracheal and nasal)	23.7-27.0	2.3-3.2	Kasavina, Zenkevich, et al. [1961]
Cartilage	24.1-31.1	2.4-3.2	Meier and co-workers [1956]
"	26.3	–	Muir [1956]
Cartilage (tracheal)	28.07	2.13	Rodgers [1951]
"	23.55	2.15	Einbinder and Shubert [1950]
Cartilage	28.7	3.3	Braint [1958]
"	22.4-26.0	5.0-6.4	Matthews and Louzeitl [1958]
Suckling rat skeleton	20.4	2.86	Dzivatkovskii [1956]

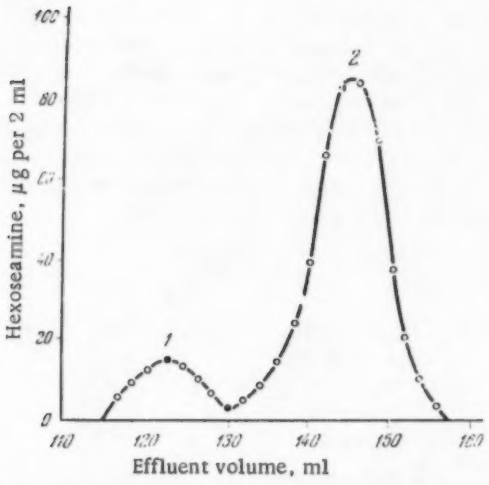

Fig. 1. Division of chonsuridum on an ion exchange membrane Dowex-50 into glucosamine (1) and galactoseamine (2).

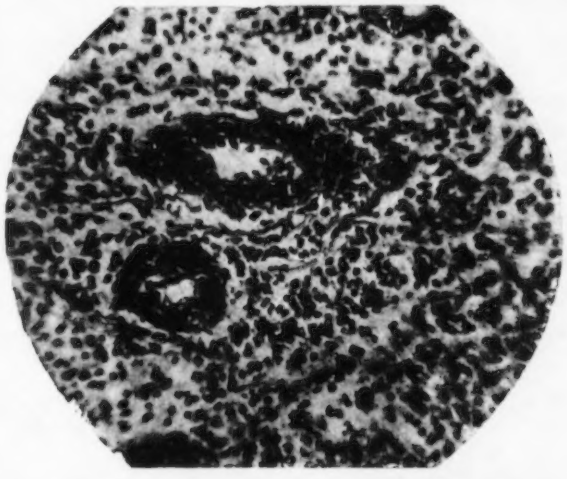

Fig. 2. Collagenous fibers around the vessels after the third day of treatment with chonsuridum (patient B); stained with azan; magnification 500 ×.

method of Niazia and Steit as modified by Larskii [3]. These indices, which indicate an inflammatory reaction very sensitively, remained within normal limits during recovery.

An attempt was made in clinical cases to study the means whereby the preparation influences the healing of wounds, and histological, histochemical, and cytochemical methods were applied. Before the preparation was used, only occasional precollagenous and collagenous fibers could be observed distributed around the capillaries in the collagenous tissue (Fig. 2). There was only a very small amount of ribonucleic acid in the endothelial cells; in other cells of the connective tissue there was no ribonucleic acid.

After 1-3 dressings with chondroitin sulfatehad been applied (on the 3rd to 10th days), the granulations contained a considerable number of capillaries which were directed toward the surface of the wound. There was an increase of connective structures between the vessels. The amorphous intercellular substance was markedly metachromatic. In it, fine precollagenous and collagenous fibers appeared; a large number of these fibers were also seen around the capillaries (Fig. 3).

The cells of the connective tissue showed a strong ribonucleic acid reaction.

Before healing, the granulation tissue was noticeably compact (the walls of the vessels were thickened), the amount of intercellular amorphous substance was reduced, it was less metachromatic, and there was a further increase in the amount of precollagenous and collagenous fibers. The connective tissue cells (fibroblasts, fibrocytes, and histiocytes) showed a less active ribonucleic reaction.

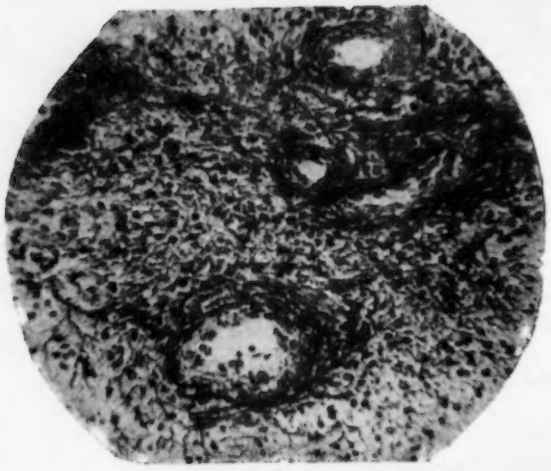

Fig. 3. Massive formation of collagenous fibers around the vessels and in the ground substance of the connective tissues after ten days' treatment with chonsuridum (patient B); stained with azan; magnification 500 ×.

A control wound treated with testicular and streptococcal hyaluronidase showed clearly that both hyaluronic and chondroitin sulfuric acid are present in the granulation tissue.

The results of these investigations show that local clinical application of chondroitinsulfate stimulates the regeneration of connective tissue cells and aids the synthesis of the proteins of precollagen and collagen. Ribonucleic acid plays an important part in this process.

SUMMARY

A study was made of the effect of acid mucopolysaccharide chondroitinsulfate on the healing of wounds. A standard preparation (chonsuridum) was tested for electrophoretic mobility, for turbidity and hexoseamine content and by chromatographic separation on ion exchange resins. The preparation was found to stimulate regeneration in slowly healing wounds. The action of chondroitinsulfate was studied histochemically. Local (external) application stimulated cellular connective tissue regeneration and the synthesis of precollagen and collagen proteins. Ribonucleic acid plays an important part in this process. The best clinical results were obtained in treating slowly granulating wounds after surgical intervention, decubitus ulcers, trophic ulcers etc.

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MULTIPLICATION OF CAPILLARIES IN DIFFERENT PARTS OF THE BRAIN IN THE POSTNATAL PERIOD

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The development of the capillary network of the brain in man and many animals is not complete until the postnatal period. There is a vigorous formation of capillaries in various parts of the nervous system for a considerable time after birth, when the capillary network, and particularly its venous portion, become finally differentiated.

The increase in the number of capillaries by branching was discovered by B. N. Klosovskii by using his own method of impregnating the walls of the vessels with silver. He and his co-workers [3,4,7-10,12,13,14] described in detail the different stages in the growth of the cerebral capillaries, and the change in the rate of growth in the cerebral cortex in various animals at various developmental stages. A new capillary is formed from a cell of the adventitia which passes along the vessel, and it passes through a number of stages before a functional capillary is formed. The earliest stage is called the "bud" stage, then as it grows it becomes transformed into a "polyp," and finally forms a "hydroid."

At all stages of the growing capillary, at the free end where the nucleus which formed its cells lies, there are numerous fine protoplasmic outgrowths. As it grows, the newly-formed capillary reaches another functional capillary through which blood is passing and becomes joined to it, usually by one or two protoplasmic outgrowths. Next the remaining outgrowths disappear, and the newly-formed capillary begins to function. Multiplication of the capillaries in the brain is largely complete in cats and dogs by the 4th-5th month, and in rats by the 2nd month of life [5,6,9,12,13,14]. In some cases we observed the appearance of solitary growing capillaries in different parts of the brain of adult rats [2]. The most intensive multiplication of capillaries in the nervous system takes place in the first few days and weeks after birth.

In the investigations referred to above, the growth of the capillaries was studied in some part of the brain either under normal conditions, or when influenced by various means. Nevertheless, no account was taken of the rate of increase of the capillaries in different parts of the nervous system and at different periods, so it is not known whether multiplication of the capillaries takes place simultaneously at the same rate throughout the brain, or whether each part has its own period at which capillary growth is maximal and corresponds to its structure and function. The object of the present investigation has been to solve these problems.

METHOD

A study was made on capillary growth in the brain of rats and cats which ranged from newborn animals to those 2-3 months old. In all, 18 cats and 70 rats of various ages were investigated. The most detailed work was done on the rat brain. The animals were killed by decapitation without anesthesia, and always at the same time of day. The brain was fixed in 10% formalin, and after fixation it was treated by B. N. Klosovskii's method [9]. Frontal serial sections 150 μ thick were made. The capillaries were counted under a magnification of 400 $\times$. The counts were made of the capillaries in different stages of growth, in 100 fields of view. In the rat brain, the growing capillaries were counted in 500-1,500 fields of view, while in the cat brain the number of fields was 1,000-11,000. Capillaries were counted in the frontal, parieto-temporal and occipital regions, in Ammon's horn, the nucleus caudatus, in the thalamus, and in the brain stem and cerebellum.

RESULTS

Table 1 shows the results of a count of newly-formed capillaries in the rat brain at different ages.

TABLE 1. Number of Newly-Formed Capillaries in the Rat Brain at Different Ages

Age of animal	Cortex			Ammon's horn	Nucleus caudatus	Thalamus	Brain stem	Cerebellum
	frontal region	parieto-temporal region	occipital region					
Newborn	126	160	72	305	501	323	184	44
7 days	170	218	162	293	269	174	144	81
10 days	385	443	149	250	212	176	—	—
15 days	288	283	449	171	113	94	—	—
18 days	68	58	53	11	—	5	7	17

It can be seen from Table 1 that in newborn rats the maximum number of growing capillaries is found in Ammon's horn, in the nucleus caudatus, and in the thalamus. In the sensory and motor cortex the number of growing capillaries is at this time much smaller than it is in the subcortical structures, but almost twice as great as in the visual cortex.

In 7-day, and particularly in 10-day, rats the rate of increase of capillaries in the subcortical nuclei and in the thalamus falls; in the cortex of the frontal and parieto-temporal regions it increases by the 10th day by $2\frac{1}{2}$ - 3 times. In the occipital cortex, the number of newly-formed capillaries at this time is also increased, but less than in the regions just mentioned. By the 15th day of life, in the frontal and parieto-temporal regions, there is some reduction in the rate of capillary increase, which is particularly marked in the parieto-temporal region. There is a great increase in the occipital region. As compared with newborn animals, the increase is six times or more. At this age the rats begin to open their eyes, so that the nerve cells of the visual centers become active.

Even by the 18th day after birth, the rate of growth of capillaries in all parts of the brain falls sharply. In the subcortical areas only occasional growing capillaries are found, and in the cortex and cerebellum they are somewhat more numerous. After 20-24 days, the number of capillaries in the cortex is 25-40 per 100 fields of view. At this time most of the newly-formed capillaries are to be found in the cerebral and the cerebellar cortex. In rats older than $1\frac{1}{2}$ - 2 months, new capillary formation has almost ceased in all parts of the brain. In other adult rats, in some cases we have observed the appearance of occasional growing capillaries, but the reason for their presence has not yet been explained.

TABLE 2. Number of Newly-Formed Capillaries in the Brain of Cats at Different Ages

Age of animal	Cortex			Nucleus candatus	Thalamus	Midbrain	Pons	Cerebellum
	frontal region	parietal region	occipital region					
8 days	77	105	82	80	33	—	—	—
23 days	131	79	83	—	34	42	82	32
30 days	149	99	112	—	—	—	—	—
32 days	254	183	156	—	—	—	—	—
35 days	37	47	95	28	33	52	12	29

New capillary formation in different parts of the brain and the occasion for its maximal intensity is closely associated with myelinization of the nerve fibers in the cortex of the different analyzers. Thus, it is known that in the cortex, the nerve fibers of the cutaneous and motor analyzers are the first to become myelinated, and the same applies to the cortical centers of the vestibular receptors. Myelinization occurs later in the visual analyzer [3,15,16]. It should be noted that the increased multiplication of the capillaries heralds the myelinization of the corresponding cortical regions.

There is a similar irregularity in the formation of new capillaries in the different parts of the brain in cats. Table 2 shows a few examples of a count of the number of growing capillaries in different parts.

Not only is there a difference in the number of newly-formed capillaries between widely separated parts of the brain, but there may be differences in a single section between functionally distinct cortical areas, and between different thalamic or midbrain nuclei. For example, in a 30-day-old kitten, in a section passing through the motor cortex, 37 capillaries per 100 fields of view were found around the sulcus cruciatus, while in the posterior sigmoid gyrus there were 30, in the gyrus limbicus anterior there were 59, and in the gyrus sigmoid anterior only 5.

The difference in the number of growing capillaries between neighboring gyri is particularly noticeable in the older animals, and it is better shown in cats.

In rats, the distribution of newly-formed capillaries in the cerebral cortex shows less difference between adjacent areas. But here too there are differences in the rate of increase; for example, between Ammon's horn and the adjacent parieto-temporal region, at certain periods the difference may be two-fold or more. In 10-day rats in the parietal cortex and on the medial surface of the hemispheres (limbic region) 283 growing capillaries per 100 fields of view could be counted, and on the same section on the convex surface of the hemispheres the count was 536, and in the nucleus caudatus it was 207.

The number of newly-formed capillaries per 100 fields of view varies greatly from one animal to another. Thus, in one newborn rat there might be 158 capillaries per 100 fields of view, and in another 271. However, as a rule, the changes occurred in all parts of the brain, and the increase was not confined to particular regions.

In healthy rats and cats there is a more or less definite and characteristic relationship between different kinds of growing capillaries. Usually the "hydroid" forms preponderate, and next in order are the "buds"; the least frequent type is the "polyp." The capillaries which are least frequently found are those which are joined to others which are already functional. A small percentage are atypical, having atrophic protoplasmic outgrowths, or a greatly constricted or twisted foot. The distribution of the growing capillaries in stages is illustrated by the following figures which applied to the brain of a newborn rat and a 30-day-old cat. Figures represent counts per 100 fields of view.

Stage	Newborn rat	30-day cat
"Buds"	76	7
"Polyps"	40	11
"Hydroids"	96	44
Various	5	1

As age increases, besides an increase in the total number of newly-formed capillaries, there is a very noticeable reduction in the number of "bud" stages, as a result of which the "hydroid" form comes to preponderate. Usually, a large number of "bud" stages is characteristic of the period of most intense multiplication ("explosive" growth of capillaries).

In animals which are newborn or a few days old, capillary growth in the brain shows several characteristic features. Protoplasmic outgrowths of the growing capillaries of newly-born animals are short, and usually few in number. The growing capillaries themselves have a thickened foot, which is apparently associated with a general expansion of the vascular-capillary net in the brain caused by the influence of the birth process itself. In different animals, the number of growing capillaries in the brain immediately after birth varies over a wide range. Even one day after birth, the newly-formed capillaries usually are longer and have a larger number of protoplasmic outgrowths.

In rats, and to a smaller extent in cats, in the first few days after birth it can be seen that capillaries grow out not only from branches of capillaries having a diameter of 4-7 μ, but also from the larger vessels whose diameter is 15-20 μ. Sometimes two or three neighboring buds join and give rise to a branch whose foot is 2-3 times greater in diameter than are the surrounding capillaries (see figure). Evidently such a branch will represent the origin of a vessel larger than a capillary.

In rats aged 13-18 days and more, and in cats after the 20th day, capillary vessels can be seen growing near nerve cells or near their outgrowth. This effect was usually noticed on preparations where the vascular capillary plexus and isolated nerve cells with all their outgrowths were impregnated together. As a rule, there are only a small number of growing capillaries in the white matter. The increase in the density of the vascular capillary

Examples of the growth of capillaries by branches from large vessels. Brain of rat aged 7 days. Impregnation by Klosovskii's method. Magnification 600 ×.

plexus of the brain in the postnatal period should be associated not only with an increase in brain size, but also with the active function of the nerve cells themselves. The latter occurs chiefly after birth, and is shown by E. N. Kosmarskaya's experiments [11]. She found that an indication of increased function in various receptors, for example the vestibular receptor, in the first few days or weeks after birth leads to hypertrophy of the bodies of the nerve cells and to an increase in the density of the vascular plexus of the corresponding nuclei in the brain stem.

Besides these general features of the growth of the vascular capillary plexus which takes place by branches, other processes also take place in the cat and rat brain, and they run their course usually during the first few days after birth. In the rat the multiplication of the capillaries is much more rapid at this time in all parts of the brain. Also, in the rat the growth of capillaries from the large vessels is also better shown, and in the first few days of life the growth of vessels by branches penetrating into the pia mater, particularly in the region of the cerebellum, can be observed. Possibly these differences are due to the fact that rats attain full maturity sooner (they are sexually mature by the second month, whereas cats are not mature until the sixth month). Probably the very rapid development of the vascular capillary plexus in the rat brain is associated with this rapid structural and functional development.

Thus, studies of the vascular capillary cerebral plexus in animals of different ages has shown that in different parts of the brain and also in different parts of the cerebral cortex, the maximal rate of capillary development occurs at different times, and the effect is to be attributed to the different periods of morphological and functional maturation of the various parts. The different rates of multiplication of the cerebral capillaries, even in parts of the brain which are not far removed from each other, indicates that apparently there are local factors of some kind which facilitate their multiplication in a particular area. Further specially directed studies will be required to determine the nature of these factors, how they are associated with the development of nerve cells, with the myelinization of the nerve fibers, and particularly with the development of oxidative enxyme systems.

SUMMARY

The brain was impregnated with silver by B. N. Klosovskii's method, and the newly-formed

capillaries were counted in serial sections. In rats and in cats it was found that both in different parts of the brain as well as in different cortical areas, the majority of new capillaries are formed by way of offshoots which develop at various times postnatally. Their formation is associated with the functional and morphological maturation of the corresponding nerve centers. Capillary branching ceases first in the brain stem and in the subcortical areas; after birth the process continues longest in the cerebral and cerebellar cortices.

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CERTAIN DATA ON THE NATURE OF DEVELOPMENT OF THE PATHOLOGICAL PROCESS IN THE GASTRO-INTESTINAL TRACT FOLLOWING Ce^{144} ADMINISTRATION

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The study of the condition of the gastrointestinal tract under the effect of radioactive substances on the organism is of interest in connection with the possibility of elicitation of certain peculiarities of its reaction and ascertaining its dependence on the character of distribution of isotopes in the organism.

The aim of the present work was to investigate morphological changes in the stomach and intestines in radiation sickness, induced via introduction of the Ce^{144} isotope, and the comparison of these changes with the lesions which originate upon the exposure to the external action of x-rays.

Ce^{144} is a β and γ emitter (β radiation equal to 0.3-0.7 mev, and γ radiation equal to 0.03-0.13 mev) with a half life of 282 days. Upon entering the organism, Ce^{144} is retained mainly in the liver (55%) and skeleton (25%). It is poorly absorbed in the gastrointestinal tract (about 0.5% according to the data of Hamilton [6] and Yu. I. Moskalev [1]), and upon oral introduction it causes mainly a local injury to the digestive organs [2].

METHOD

Experiments were conducted on 30 white rats to which Ce^{144} had been administered via gastric sound at the rate of 12-15 μC/g of body weight. The examination was carried out at periods from one to 30 days. The material was fixed in a 10% solution of neutral formalin and placed in celloidin and celloidin-paraffin. The histological slides were stained with hematoxylin-eosin, mucicarmine according to Meyer, and picrofuchsin according to van Giesen. In addition, the mitoses in the epithelium of the crypts of the small intestine were counted and the isotope distribution in the gastrointestinal tract was studied by the method of autoradiography.

RESULTS

Following administration of Ce^{144}, the animals manifested a liquid stool, sluggishness, thirst, anorexia, loss of weight, and emaciation. Death usually occured in the 3rd-4th week from the beginning of the experiment. At the autopsy of animals killed or perished within the first five days an atony of the stomach and large intestine was observed. In all examined rats, a liquid bloody gastric content was noted as well as an enlargement of the lymph nodes of the mesenterium; in the cecum, sigmoid and, less frequently, the distal section of the jejunum, small focal necroses were found covered with purulent secretion; sometimes adhesions with the loops of the small intestine were observed. Also dryness of the serous membranes, liver degeneration, and at times pulmonay hemorrhages.

Upon microscopic examination of the stomach, it was found that in the majority of rats there were infiltrates in the basal section of the mucosa and the submucous layer which consisted of lymphoid cells and, less frequently, of eosinophile and neutrophile leucocytes. The submucous layer in some animals was hypertrophied. In some rats killed at the end of the 4th week after the isotope administration, no morphological changes in the stomach were observed.

In the jejunum during the first five days from the start of the experiment, a reduction of mitotic activity of the mucous membrane epithelium by approximately 47% of the initial level was observed. In the crypts, in the stroma of the mucosa, and in the lymphatic follicles of Peyer plaques, products of cellular disintegration were encountered which were incorporated by macrophages. In addition, during the first three days, a considerable swelling of the nuclei of the crypt epithelium took place.

In rats killed on the 4th-5th day, in addition to changes in the structure of the basal sections of the mucous membranes, considerable changes were found in the cilia, which appeared shortened and covered with flattened

epithelium containing large vesicle-like nuclei. Scanty beaker-like cells were enlarged and had a cricoid form. The mucosa was hyperemic and a large number of lymphoid cells were present in its stroma.

On the 7th day a gradual restoration of the mucose structure began to take place. Together with crypts lined with flattened epithelium and containing products of cellular disintegration, crypts appeared of the usual structure and the number of mitoses rose to the initial level. The ciliary epithelium reverted to its usual form. Within 10 days after the administration of Ce^{144}, the epithelial structure of the mucosa was fully restored. During this period only an increase in the number of lymphoid cells and a reduction in the size of lymphatic follicles of Peyer plaques could be noted.

In the iliac epithelium a consecutive development of morphological changes analogous to the disturbances in the jejunum was observed. Microabscesses were encountered in the mucosa. The submucous layer and mesenterium in many animals became infiltrated with neutrophile leucocytes and lymphoid cells. More pronounced changes occured in the distal end of the colon, where necroses and ulcers of the mucosa were found.

The large intestines were the most affected; as early as within 24 hours following administration of the isotope, a considerable destruction of the epithelium was observed, and on the 2nd-3rd day extensive necroses and ulcers of the mucosa appeared (Fig. 1). The epithelium of areas adjacent to the ulcers was in a state of dedifferentiation. Microabscesses were present in the crypts. A large number of leucocytes were present in the granulation tissue which filled the bottom of the ulcers. Within $1\frac{1}{2}$ - 2 weeks after administration of the isotope, in addition to the above-mentioned cellular forms, isolated fibroblasts began to appear in the granulation tissue; later, within three weeks, macrophages and eosinophile leucoytes appeared, and formation of collagen fibers was observed. The number of newly formed vessels was slight. A considerable quantity of fibroblasts and collagen fibers was found also in the mesenterium of the large and, sometimes, the iliac intestines.

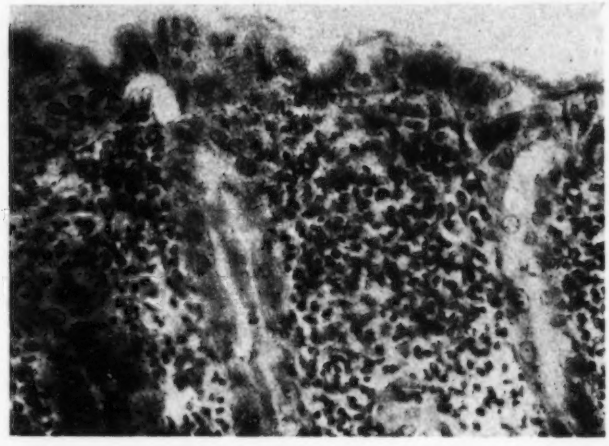

Fig. 1. Destruction of the epithelium of the mucosa of the large intestine of a rat and an increased quantity of cellular elements in the stroma, 24 hours after the introduction of 12 $\mu C/g$ of Ce^{144}. Photomicrograph. Stained with hematoxylin-eosin; magnification 200 ×.

Microabscesses were sometimes found in the newly formed mucous membrane at the edges of ulcers (Figs. 2 and 3).

In some rats killed or perished within 3-4 weeks after the affection, a focal hyperplasia was observed of the mucous membrane as well as formation of displaced and distended crypts. The vessels of all sections of the intestinal tract had thickened, swollen walls and the lumen of the large arteries was constricted. Many neural cells of the Auerbach plexus situated in the ulcerated areas stained intensively with hematoxylin or had the appearance of dark clots. Lymphocytes and some lymphostasis were found in the enlarged lymphatic nodes of the mesenterium, and in some cases foci of myeloid hemopoiesis were observed in the lymph nodes.

Upon autoradiographic examination, the largest amount of the isotope was elicited in the smears of the contents of the large intestine during the first 48 hours after the isotope administration. The activity of the wall of the stomach and small intestine, apparently on account of isotope absorption, proved to be somewhat higher when compared to the activity of the wall of the large intestine. Subsequently, during the entire period of investigation, the highest Ce^{144} content was found in the liver.

The obtained results indicate that the affection of the gastrointestinal tract following a single oral administration of Ce^{144} had a nonuniform character. The morphological changes increase from the proximal to the distal

Fig. 2. Ulcer of the large intestine on the 23 rd day after the introduction of 12 μC / g of Ce^{144}. Photomicrograph. Hematoxylin-eosin; magnification 15 ×.

Fig. 3. Microabscesses in the regenerated mucosa of the large intestine in a rat on the 23rd day after the introduction of 12 μC/g of Ce^{144}. Photomicrograph. Hematoxylin-eosin. Magnification 70 ×.

end of the gastrointestinal tract, the greatest impairment taking place in the large intestine.

The stomach shows a mild inflamatory reaction, which in some animals disappears toward the end of the 1st month.

The involvement of the jejunum presents the picture of a radiation burn and is similar to the lesions observed following external irradiation [4,5,8,9]. As in the case of external irradiation, the changes are most pronounced in the crypts, and later in the cilia. A period of reparation follows the phase of destructive changes and, approximately within 10 days, the epithelial structure of the small intestine becomes fully restored. However, in contrast to the effect of external irradiation the morphological changes following internal administration of Ce^{144} proved to be less marked and more prolonged. The greatest changes following direct total irradiation with x-rays usually manifest themselves in the small intestine, whereas during the early stages after Ce^{144} administration the large intestine shows greater pathological changes with the formation of extensive necroses and ulcers accompanied by a prolonged inflammatory process. In addition, in the small intestine, especially in its distal section, there appear phenomena of inflammation after Ce^{144} introduction which are absent in the case of total x-ray irradiation. The leucocytic reaction, generally absent in x-ray irradiation, is well marked on Ce^{144} administration.

Thus, the radiation injury is combined in this case with the picture of an acute inflammation. The insignificant impairment of bone marrow hemopoiesis in peroral administration of Ce^{144} is, apparently, one of the basic reasons responsible for the presence of a large number of leucocytes in the tissues.

This unique affection of the gastrointestinal tract depends on the physicochemical properties and the method of introduction of the isotope.

As we know from the data in the literature, Ce^{144} is absorbed in very small quantities (0.05%) in the gastrointestinal tract and is excreted basically within 1-2 days after its introduction. The faster movement of the contents along the small intestines causes a lesser injury to this section, whereas the detention of food in the iliac and especially in the colon apparently represents the basic cause of the radiation burn of the distal segment of the digestive tract. Thus, the basic reason for the development of mosphological changes is, presumably, the direct effect of radiation on the structures of the gastrointestinal tract.

The results of autoradiographic examinations and toxicological data permit us to assume that upon introduction of the isotope the intestines are basically affected as the result of a relatively short action of massive radiation doses.

The obtained data correspond to the scanty reports in the literature which mention the peculiarities of the reaction of structures of the gastrointestinal tract, depending on the properties of the radiation action. Thus, Hulse

[7] reports the emergence of an inflammatory reaction and microabscesses in the intestines following irradiation of the abdomen with β-particles of Sr^{90} in a dose of 9400 rad. M. V. Svyatukhin [3] describes the development of fibrinous necrotic colitis under conditions of peroral administration of a mixture of poorly absorbed β-emitters, and assumes that the basic role in the development of morphological changes is due to the local radiation effect. Sommers and Warren [10] note the formation of abscesses in the large intestine and distal part of the small intestine in parabiotically united rats following irradiation of one of them with a 400-1600 r dose.

SUMMARY

A study was made of morphological changes occurring in the gastrointestinal tract of rats after Ce^{144} administration(12-15 μC/g of body weight). Destructive and necrobiotic changes in the mucosal epithelium accompanied by inflammatory reaction were characteristic of gastrointestinal tract lesions. The degree of these changes increased toward the distal end of the digestive tract, the large intestine being most affected.

After external x-ray irradiation and Ce^{144} administration, certain features specific to the reaction of the gastrointestinal tract could be observed. These depended on the physicochemical properties and the method of isotope administration.

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METHODS OF DETERMINING SPECIFIC AND NONSPECIFIC CHOLINESTERASE IN MICROVOLUMES OF BLOOD

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Recently many reports have been made of the estimation of the cholinesterase activity of the blood serum and red cells. More than 100 variations of methods of determining cholinesterase chemically in the blood have been published, in addition to a large number of articles dealing with the rate of enzyme activity in health and in desease. The considerable effort made to work out simple and sufficiently precise methods has been stimulated not only by the importance of cholinesterase as a transmitter of nervous impulses, but because of the growing importance of determining its activity in a number of clinical conditions [2,6,7,8,10,13,16,18,19,22,23,24]. It must also be remembered that cholinesterase activity may be supressed by means of various toxic substances, some of which are being increasingly applied in agriculture as insecticides containing phosphorus [17].

Unfortunately, most of the methods designed for the clinical determination of cholinesterase activity in the blood take account only of the nonspecific cholinesterase of the serum [1,4,9,11,12,14,20]. However, the necessity for obtaining blood serum makes it less easy to carry out a series of observations, both because rather large quantities of blood must be obtained by venipuncture, and because time must be spent in separating the serum. When working with small laboratory animals such as mice, rats, frogs, etc., repeated estimations of serum cholinesterase activity cannot be made, for the reasons already given.

Methods for the determination of cholinesterase in the erythrocytes of whole blood are much more complex and laborious [5,15,21].

These considerations have led us [3] to propose a comparatively simple colorimetric method for determining cholinesterase activity in a drop of blood. However, the method suffered from several defects; the color changes were not sufficiently sharp, which made it difficult to determine the end point, and it was not possible to separate the specific and nonspecific cholinesterase activities.

The purpose of the present communication is to describe a very much more precise and simple method for the differential determination of specific and nonspecific cholinesterase activities, which may be applied to a few microliters of blood. In developing the method we have attempted to stay close to the simple blood analysis which is well known in pathological laboratories.

In order to work out ways for the differential determination of the specific and nonspecific components, we studied the rate of enzymatic hydrolysis of equimolecular solutions of the different esters by the cholinesterase of the blood serum and of the erythrocytes (Table 1).

TABLE 1. Rate of Hydrolysis of Equimolecular Solutions of Choline Esters by the Cholinesterase of the Blood Serum

Ester	Time for 50% hydrolysis		Ratio of time of hydrolysis of ester to that of aceylcholine
	Mean (of 10 determinations	Variation range	
Acetylcholine	8.0	6.0-10.0	1.0
Acetylthiocholine	7.5	6.0-9.0	0.94
Butyrylcholine	4.0	3.5-4.5	0.50
Butyrylthiocholine	4.5	3.5-6.0	0.56

It can be seen from Table 1 that butyrylcholine is hydrolized by the nonspecific cholinesterase of the serum twice as rapidly as is acetylcholine; it is also known that the rate at which butyrylcholine is broken down by the specific cholinesterase of the erythrocytes is extremely slow, so that this quantity may be neglected.

We have developed three variants for the method of determination of cholinesterase activity which can be applied to comparators for droplet reactions and for reactions in test tubes and in blood count pipettes. In all three arrangements, the principle for the determination of the enzymatic activity is to determine the time required to break down 50% of the choline ester taken: for the determination of nonspecific esterase the choline used is butyrylcholine, while acetylcholine serves for the determination of specific and nonspecific esterases.

The duration of the reaction is measured from the rate of change of the color of an indicator in a standard buffer solution, caused by the liberation of acid through the hydrolysis of esters. The colorimetric comparison is made by using standard solutions whose color corresponds to the initial and final color of the reactive mixture. To mask the scarlet color of the blood, a rather concentrated solution of bromthiol blue is used, whose color changes from blue to yellow over a pH range of 7.6 to 6.0.

Reagents Require

1. Principal buffer indicator solution. Two hundred mg of bromthiol blue indicator are triturated in a mortar with 20 ml of 0.1 N solution of caustic soda. The blue solution is poured from the mortar into a measuring flask. The mortar is washed out several times with small amounts of water free from carbon dioxide, and the washings are added to a 1,000 ml measuring flask. Fifty ml of 0.1 M solution of boric acid (in a 0.1 M solution of potassium chloride) and 5 ml of a 5% saponin solution are then added. The contents of the flask are made up to the mark with distilled water free from carbon dioxide. The solution so obtained is of a saturated blue color, and has a pH of 8.4.

2. Buffer-indicator solution for preparing colorimetric standard No. 2. A mixture is made of 9.75 ml of the principal solution No. 1 with 0.25 ml of 0.1 N hydrochloric acid. The solution has a saturated yellow color and a pH of 3.5. The solution is stable.

3. Buffer-indicator solution of butyrylcholine for determining nonspecific cholinesterase. A mixture is made in a small beaker of 1.8 ml of the principal solution No. 1 with 0.2 ml of 1.57% of butyrylcholine (or 1.1% solution of butyrylcholine chloride). This solution, like solution No. 4, must be prepared not more than three hours before it is used.

4. Buffer-indicator solution of acetylcholine for determination of specific and nonspecific cholinesterase. A mixture of 1.8 ml of principal solution No. 1 with 0.2 ml of a 1% solution of acetylcholine is made in a small beaker.

To carry out the reaction in Vidalevskii test tubes and in blood count pipettes for incubation at 40°, a plastic thermostat comparator was made. It consisted of a rectangular box made of pieces of plastic stuck together, and containing a stand made of white plastic for holding test tubes or blood count pipettes.

When carrying out the reaction by the droplet method, we used the comparator which we had developed ourselves for this reaction. The principal feature of the comparator was a disc of colorless plastic 7-10 mm thick with recesses drilled in it of uniform depth and diameter. For most of the tests we used recesses having a 5 mm diameter and a depth of 8 mm, which held about 0.15 ml of fluid. They were arranged in two or three rows at an equal distance from each other (Fig. 1). The second important feature was the screen having a black and white surface. It served as a base for the comparator, and was connected with the disc mentioned above by a spring arm which enabled the relative position of the disc and screen to be changed by a rotation, so that a uniformly illuminated background was obtained. The black surface could be changed for a white by rotating the arm through 180°.

In order to enable samples to be placed in the thermostat, a construction was arranged which made it possible to move the plate containing the recesses within the plastic thermostat comparator described above (Fig. 2).

Method of carrying out the reaction. After the skin of a finger has been punctured, 20 microliters of blood is taken (the volume of one pipette for collecting hemoglobin) and it is blown into a test tube with 0.4 ml of distilled water. If necessary, the operation may be interrupted for 3-5 hours at this stage. When a determination is to be made in the recesses of the comparator for droplet reactions, by means of calibrated pipettes (calibrated to give drops of a standard size), two drops of hemolyzed blood are introduced, and the comparator is placed for 5-10 minutes in a thermostat containing water at 40°, in order to equalize the temperature. Then three drops

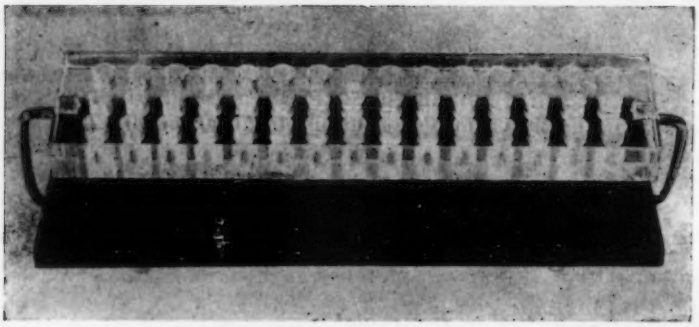

Fig. 1. Comparator for droplet reactions

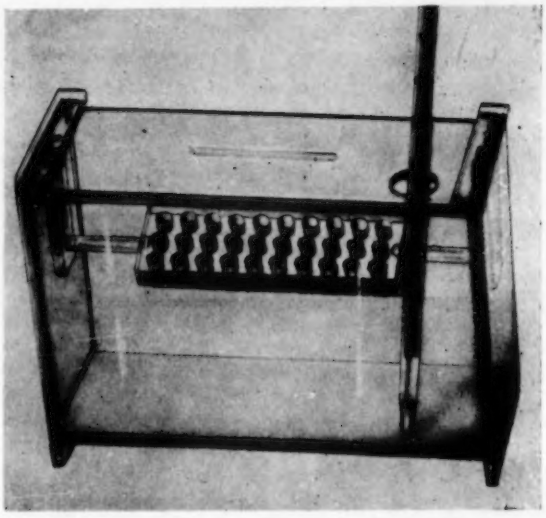

Fig. 2. Arrangement of the comparator for droplet reaction within the thermostat.

of solutions Nos. 1,2,3 and 4 are introduced into the appropriate compartments. The contents of the recesses are stirred up with fine glass rods (sealed-off capillary tubes) and the time of the onset of the reaction is noted. In this way two colorimetric standards are obtained, and two samples tested for the determination of specific and nonspecific cholinesterase. The color of the contents of the first recess, into which solution No. 1 was introduced, corresponds to the initial color of the reaction mixture (standard No. 1). The contents of No. 2 recess, into which solution No. 2 was introduced, has a pale brown color, which corresponds to the color given by a sample in which 50% of the substrate is broken down (standard No. 2). In the third and fourth recesses, to which solutions No. 3 and 4 are added, there is an enzymatic breakdown of acetyl- and butyrylcholines. The rate of change of color of these recesses away from the second standard indicates the activity of the specific and nonspecific cholinesterases in the blood under test. The time taken for the equalization of the color of the two samples (with acetyl and butyrylcholines) is found from the colorimetric standard No. 2. Colorimetric comparisons are carried out without removing the comparator from the thermostat, and are made against the white background of the plastic base plate.

When the determination is made in test tubes, the tubes are placed in the holder of a thermostat. To each tube 0.1 ml of hemolyzed blood and 0.5 ml of water is added. To equalize the temperature, the stand is placed for 5-10 minutes in a thermostat containing water at 40°. Then 0.15 ml of solutions Nos. 1,2,3 and 4 is added to each of four tubes. As a result, as in the previous method, two colorimetric standards are obtained, one of which corresponds to the original (blue-green) color, and the second to the recorded (yellow-brown) color of the reaction mixture formed after breakdown of 50% of the substrate. The contents of the tubes are carefully mixed, and the time of onset of the reaction noted. The time is taken at which equalization of the color of the samples with standard No. 2 occurs. The colorimetric comparisons are made without moving the test tubes from the thermostat, and are made against the white background of the back wall of the stand.

Calculation of cholinesterase activity. In solutions No. 3 and No. 4, choline ester is present in a concentration of 0.005 M, and in standard solution No. 2, acid is introduced at a concentration of 0.0025 M, which corresponds to a breakdown of 50% of the substrate. To compare the reaction mixtures, two volumes of hemolyzed blood are mixed with three volumes of the solutions. Because the blood is diluted 1:50 times, and a concentration of the substrate is 0.003 M (or 1.5 micromoles per ml for 50% breakdown), the equation may be written as follows:

$$A_{ch} = \frac{1.5 \cdot 50}{T} = \frac{75}{T}.$$

where Ach is the activity of the blood cholinesterase expressed in micromoles of choline ester broken down per ml of blood per minute. In calculating the activity of the specific (A_1) and nonspecific (A_2) cholinesterase, it must be remembered that butyrylcholine is broken down by the nonspecific cholinesterase of the blood serum 100 times more actively than by the specific cholinesterase of the erythrocytes. Therefore, the value for the enzymatic activity obtained by estimation with solution No. 3 (A_3) indicates the total activity of both enzymes. In other words, A_3 (solution No. 3) = A_1 (cholinesterase erythrocytes) + A_2 (cholinesterase serum from acetylcholine).

Note (see Table 1) that a constant equal to the ratio of the activity of serum cholinesterase (as indicated by acetylcholine) to the activity of serum cholinesterase (as indicated by butyrylcholine) is approximately 0.5. Hence, the activity of the specific cholinesterase may be calculated from the following formula:

$$A_1 = A_3 - 0.5A_4,$$

where A_4 is the activity of the nonspecific cholinesterase of the blood with respect to butyrylcholine.

It must be emphasized, however, that such calculations are very arbitrary, because other kinds of esterases may also take part in the breakdown of choline esters, and there may be considerable variation in the amount of such esters between different species of animal. It is therefore more correct to express the values obtained for cholinesterase activity purely as the number of micromoles of acetyl- and butyrylcholines broken down per ml of blood per minute (or per hour).

The methods we have described were checked by testing more than 200 healthy subjects and patients, as well as several animal species. Clinically, it was possible to demonstrate pathological conditions by applying the method to cases of damage to the liver parenchyma, where cholinesterase activity attains an extremely low value.

Table 2 shows the mean values of the cholinesterase activity of the blood of some animals and man.

TABLE 2. Cholinesterase Activity of the Blood of Some Animals and Man (hydrolysis at 38°)

Blood	Time for hydrolysis to attain standard no. 2, min		Cholinesterase activity, μM/ml/min		$K = \frac{A_3}{A_4}$
	butyrylcholine (soln. 4)	acetylcholine (soln. 3)	butyrylcholine (A_3)	acetylcholine (A_4)	
Man	18,5(11—26)	16,8(10—23)	5,7	5,9	0,97
Monkey	15,8(13—19)	23,4(21—28)	6,3	4,3	1,5
Dog	33(22—44)	55,4(46—68)	3,0	1,8	1,6
Guinea pig	16,7(12—20)	33(24—40)	6,0	3,0	2,0
Rabbit*	>3 hr	48,6(37—59)	0,5	1,0	—
Mouse	21,2(14—26)	25,2(17—29)	4,7	4,0	1,2

*Because of the low concentration of the enzyme, the volume of the buffer solutions Nos. 3 and 4 was reduced by almost half in determining the cholinesterase activity in rabbit blood.

Note: The limits of variation are indicated in brackets.

In considering Table 2, it can be seen that in different human samples, and in different samples from the same animal species, cholinesterase activity, as indicated by the hydrolysis of both acetylcholine and butyrylcholine, varies within quite wide limits.

From the average figures, and particularly for the ratio of the rates of hydrolysis of butyryl- and acetylcholines of whole blood, it can be seen that there is a real difference between the cholinesterase "spectrum" of the blood of different species.

The methods described showed to particular advantage in experiments on small animals. It was then possible to follow the change in enzymatic activity from repeated blood samples taken, for example, from the tail of a mouse. An illustration may be given from the changes in cholinesterase activity following a subcutaneous injection of 0.1 mg/kg of proserine (Fig. 3).

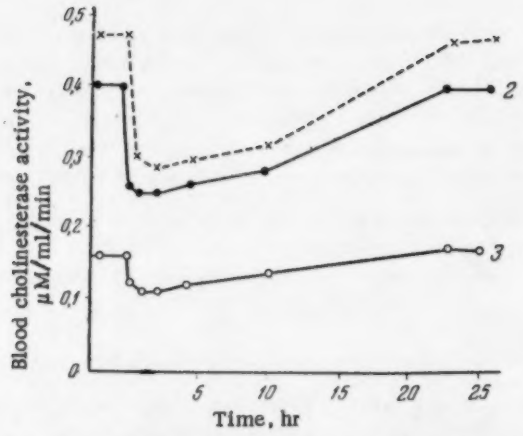

Fig. 3. Changes in cholinesterase activity in the blood of white mice which have received a subcutaneous injection of 0.1 mg/kg of proserine. 1) Rate of hydrolysis of acetylcholine (A_3); 2) rate of hydrolysis of butyrylcholine of nonspecific cholinesterase (A_4); 3) calculated rates of hydrolysis of acetylcholine by specific cholinesterase (A_1).

It is clearly seen that proserine, as a powerful but reversible inhibitor, causes a temporary suppression of both nonspecific and specific cholinesterase. By the end of the first 24 hours, the body of the mouse is completely free from proserine.

SUMMARY

A technique is described for drop colorimetric determination of the specific and nonspecific cholinesterase activities in microvolumes of blood; the method is based on changes in the hydrogen ion concentration in the reactive mixtures.

A new device makes it possible to examine a large series of samples; only a few microliters of blood are used for each test. The suggested method may also be used for repeated determination of cholinesterase activity in the blood of small laboratory animals such as mice. Mean blood cholinesterase activity values are given for various animals.

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THE SYNTHESIS OF THE SODIUM SALT OF β-OXYBUTYRIC ACID FROM THE ACETOACETIC ESTER

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Existing methods of preparing β-oxybutyric acid and its salts [2,3,5-7] do not satisfy the requirements for biochemical investigations. A disadvantage is that they require for their preparation substances which are in short supply, or else that they give very low yields. In some cases [7] the preparation of β-oxybutyric acid obtained contained toxic impurities which made it impossible to use it in biochemical experiments. By the method described below, which is simple, quite a good yield of sodium oxybutyrate is obtained, and the material from which it is produced (acetoacetic ester is readily obtainable. From 25 g of acetoacetic ester, more than 7 g of a 100% pure preparation of sodium oxybutyrate may be obtained. The purity of the preparation was tested by the method of Klingmüller and his collaborators [4]. The preparation was tested by oxidative phosphorylation of homogenates of rabbit heart muscle, and on the mitochondria of the rat liver. The principle of the method is to reduce acetoacetic ester by means of an amalgam of aluminum in moist ethyl sulfate [1] and then to saponify the ethyl ester of β-oxybutyric acid so obtained to form sodium β-oxybutyrate.

The following reagents were used: 1) commercial acetoacetic ester, redistilled in vacuum; 2) metallic aluminium turnings; 3) a 2% solution of mercuric chloride; 4) moist ethyl sulfate (commercial ethyl sulfate is washed twice with water in a separating funnel, then an equal volume of water is added, and after 15 minutes the ethyl sulfate is separated from the water, and it is then ready for use); 5) a 12% caustic soda solution; 6) absolute ethyl alcohol.

I. Preparing the aluminium amalgam. Thirty-five grams aluminium turnings are added to 200 ml of 12% sodium hydroxide, and hydrogen is liberated. After a few minutes, when the hydrogen is being evolved vigorously, the sodium hydroxide is poured off, and the aluminium turnings are washed three times in 200 ml of distilled water. Two hundred ml of a 2% solution of mercuric chloride is now added to the aluminium turnings which have been cleaned in this way. Hydrogen is then liberated, and the turnings become shiny. One or two minutes after the amalgam has formed, the turnings are quickly washed in 400-600 ml of water, when the mixture becomes hot and the hydrogen is rapidly evolved. The amalgam is prepared immediately before it is required.

II. Preparation of the ethyl ester of β-oxybutyric acid. The following substances are now added to a round-bottomed flask fitted with a reflux condenser: 25 g of acetoacetic ester, the whole of the freshly prepared amount of aluminium amalgam, and 250 ml of moist ethyl sulfate; the mixture is left to stand in a water bath at room temperature for 4-6 hours (if the flask becomes hot, it must be cooled in ice water). As the reaction proceeds, the contents of the flask increase considerably in volume, so that for some time after the onset of the reaction there may be some interference with the liberation of hydrogen. If so, up to 400 ml of moist ethyl sulfate is added. The mixture is then left overnight (if hydrogen is evolved rapidly, it is stood in a bath with ice).

On the next day, the contents of the flask are filtered and repeatedly washed on a Buchner funnel with dry ethyl sulfate. If the filtrate is turbid it must be passed through a No. 2 glass filter.

The transparent filtered ester is dried for 1-2 days over fused sodium sulfate, after which the ethyl sulfate is evaporated away on a water bath at 35°, the ethyl ester of β-oxybutyric acid is distilled and the fraction coming off between 76 and 77° at 15 mm is collected. Here it must be remembered that the boiling point of acetoacetic acid is 73° at 15 mm, and is close to that of the ester of β-oxybutyric acid.

III. Saponification of the ester of β-oxybutyric acid. To 10 ml (about 10 g) of the ester of β-oxybutyric acid which has been prepared, an equivalent amount of a 7% solution of sodium hydroxide is added.

When the ester of β-oxybutyric acid is mixed with the alkali, an oil is formed which passes into solution after 10-30 minutes. Immediately afterwards, the solution is evaporated in a vacuum at 35-40° and 15 mm pressure until a syrup remains, which crystallizes out on cooling. The crystals of sodium β-oxybutyrate rapidly volatilize in air, and they must therefore immediately be transferred to a vacuum dessicator, where they are dried over phosphorus pentoxide for 1-2 days.

IV. Purifying the sodium β-oxybutyrate. It is cleaned by boiling in absolute alcohol. Before doing so, it should be well dried, because traces of water greatly increase the solubility of the salt in alcohol. For this reason, all the reagents used in the purification process are protected from the access of atmospheric moisture.

The sodium β-oxybutyrate is placed in a flask fitted with a reflux condenser and a calcium chloride tube, and is heated with 50-70 ml of absolute alcohol on a closed stove. Then, after a short period when the mixture of salt and alcohol has become fluid, the contents of the flask appear white from the crystals which separate out. If a thick yellowish fluid is formed in the flask, more alcohol must be added until crystallization starts.

The crystals are immediately separated from the hot alcohol on a glass filter No. 1, and are rapidly washed several times with cold absolute alcohol. The snow-white flaky crystals of the sodium salt of β-oxybutyric acid are dried in a vacuum dessicator over phosphorus pentoxide.

After about half the alcohol has been driven off from the mother liquor, and it has been left for the night to cool, a further considerable quantity of crystals can be obtained, which are then cleaned as described above. The total yield of sodium β-oxybutyrate is 7.7 g, which represents 33% of the theoretical yield from the acetoacetic ester.

We would like to express our sincere thanks to É. I. Budovskii for having suggested this possible method of synthesis, and for his advice during the work.

SUMMARY

A method is described for preparing the sodium salt of β-oxybutyric acid from acetoacetic ester. The acetoacetic ester is reduced by aluminium amalgam in moist sulphuric acid, and the β-oxybutyric acid thus obtained is then saponified to form the sodium salt of β-oxybutyric acid. The yield is approximately 30%.

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ANGIOSTOMY USING AN ISOLATED SEGMENT OF SMALL INTESTINE

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A great deal of work has been done on the function of intact organs in chronic experiments. Considerable difficulties are encountered, and one of the most promising methods is that of angiostomy.

It was proposed by E. S. London [2], who used a metallic cannula. The operation is carried out in two stages: first the vessel is prepared to receive the cannula, and then it is sewn in position. However, a metallic cannula is not convenient, because it constitutes a foreign body.

In I.A. Pigalev's [3] modification, a skin fragment is used as a cannula. In our opinion, the method is still unsatisfactory. Firstly, such a cannula may close up, and any maceration of the skin will produce this effect; secondly, because of the insufficient blood supply such a cutaneous cannula may necrose.

F. D. Vasilenko [1] used an isolated segment of the small intestine as a cannula. After a portion of intestine had been isolated, one end of it was split open for a distance of 1.5 cm, after which the inferior vena cava was freed from the surrounding tissue and surrounded by the doubled portions of the intestine, which were then sewn in place. A disadvantage of the method is that it can be used only on large vessels; in addition, there is always the danger that the vessel may be compressed by the intestine, which is supplied with powerful muscle layers.

We have used successfully a new modification of the angiostomy method in experiments on 28 dogs. We followed F. D. Vasilenko in using an isolated segment of small intestine as a cannula. The method has the following advantages: the cannula cannot close up; because of the continuity of blood vessels and nerves which supply the isolated intestinal segment, it cannot necrose; the extensibility of the portion of gut enables the lumen of the cannula to be altered; the operation may be applied to various vessels; animals on whom this operation has been performed do not require any special care.

The whole of the operation was carried out at one time. To begin with, one or two portions of small intestine, depending on how many were required, were isolated without breaking the continuity of the mesenteric vessels and nerves. The two cut ends of the remaining intestine were joined. Next, a portion of mucous membrane at the oral end of the isolated segment (measuring 0.5 cm) was cleaned, three or four silk stitches were inserted in it, and it was sewn on to the wall of the vessel so as to include the surrounding tissue. A layer of mesentery was then placed around the angiostomy, and the other end of the isolated segment of small intestine was brought out through the opening in the peritoneal cavity.

In our experiments we sewed the intestinal cannulae to the portal and hepatic veins. Experiments using this method were performed on 28 dogs. It was found that the small amount of fluid which is secreted by the isolated intestinal segment does not in any way harm the vessel to which it is sewn. A dissection performed at the end of the experiment showed that the isolated intestinal segment had healed beautifully to the vessel walls.

SUMMARY

A new modification of E. S. London's angiostomy method is described. Isolated segments of the small intestine were used as cannulae. The intestinal cannulae were joined to the portal and hepatic veins in 28 dogs. The advantages of this method were as follows: the isolated intestine has the convenient property that its size may be changed at will; there is no danger of necrosis or of loss of patency, because the vessels supplying the cannula remain intact. Good unity with the vessels guarantees reliability of the results.

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A MULTICHANNEL WATER-MERCURY MANOMETER*

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Despite the wide use of electromanometers, mercury and water manometers nevertheless continue to find applications in the laboratory. However, it is difficult to make them sufficiently convenient in use, and it is not easy to design reliable devices for the simultaneous recording of several processes.

These difficulties have been overcome in an instrument which we have used for several years.

The manometric multichannel system is mounted on a vertical rod. On it are fixed three transverse members; two of them carry openings for the glass tubes of the water and mercury manometers (Figs. 1 and 2).

The diameter of the hole for the mercury manometers is 8 mm, and for the water manometers 12-14 mm. The uppermost transverse member bears apertures 1 mm in diameter to position the glass rods of the manometers, which are a close fit in the transverse members where they are held in place by soft metal screws, and no kind of packing is required.

A better method of securing the glass tubes in the apertures is to use clamps. Then, when they are tightened, the tubes are not displaced, as they may be when a screw at one side is used. Each limb of the mercury manometer is mounted separately, and then connected with a stiff rubber or polyethylene tube.

Usually single-channel water manometers are used, but to record gaseous pressure, or when air transmission is used, they also may be made in two-channel form (V-shaped).

Floats for the mercury manometers are made from plastic, and those for the water manometers from foamed plastic.

For the vertical limbs of the manometers we used thin-walled capillary tubing of about 1 mm diameter. Such a tube slips readily through the holes in the metal. The movement of the capillary is controlled at three points: its attachment to the float, the aperture in the metal cap at the output of the manometer, and the opening in the uppermost cross member.

When the whole of the kymograph paper width is used for recording, for each channel the length of the capillary tube must be greater than twice the paper width, and the distance between the upper and middle transverse members must be equal to the paper width. When the float is in the lowermost position, the capillary tubes must not move out of the openings in the uppermost transverse member.

To the capillary tubes are attached pointers made of movie film (see Fig. 2). The position of the pointers which write on the kymograph, and the pressure they exert on the paper, is controlled by a series of parallel threads which are held in tension by removable bars which are attached to the middle and upper cross pieces. Tension is applied to the threads by a portion of rubber connected between the end of each thread and its attachment. The pressure of the pointers on the paper is easily regulated by moving the threads along the bars.

The pointers are arranged so that their tips come to lie in a vertical line, while each pointer lies in a different horizontal direction (see Figs. 1 and 2). In this way, there is no interference between the vertical movements of the different pointer. In order for the pointers not to catch in each other when they are strictly aligned vertically so as to produce accurate synchronization of the record, it is important that all the ends of the pointers should be

* The metallic parts of the manometer were made in the workshop by V. M. Vlasyuk.

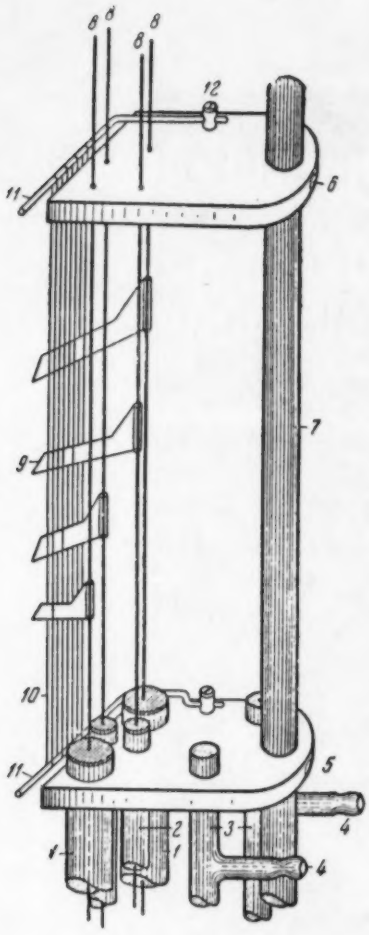

Fig. 1. Diagram of the recording portion of a four-channel water-mercury manometer. 1) Water channels of the manometer; 2) mercury channels; 3) second limb of mercury manometer; 4) connection to cannula or catheter; 5) middle transverse member; 6) uppermost transverse members; 7) vertical bar; 8) capillary tubing; 9) recording arms; 10) threads controlling position of recording arms; 11) rod to maintain tension in threads; 12) support for removable bar.

cut in one direction at an angle not less than 45°.

The pointers should be made as short as possible, and the manometer must therefore be brought as close as possible to the drum.

Some kymographs are of inconvenient shape, and it is then impossible to bring the manometer directly up against the drum. In such cases the drum diameter must be increased.

When the multichannel manometer is correctly mounted and adjusted, many advantages are obtained from its convenience in operation. The whole manometer may be removed or replaced in position by a clamp holding the vertical rod. It is convenient to be able to replace it in its previous position relative to the kymograph, and there will then be no necessity for further alignment of the points of the different channels. The pointers, being held in position between parallel threads, will then not need to be adjusted.

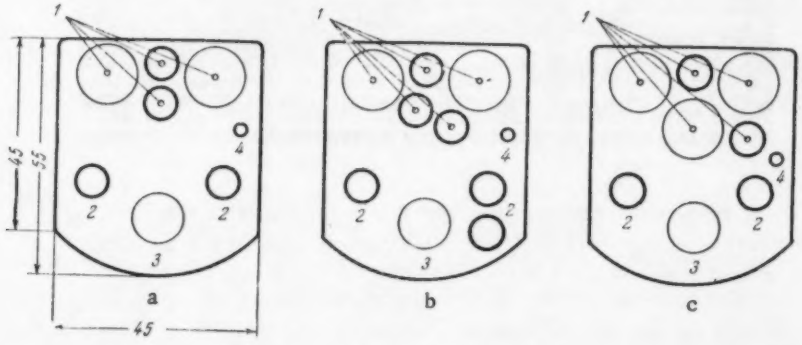

Fig. 2. Diagram of cross members: a) with two water and two mercury channels; b) with two water and three mercury channels; and c) with three water and two mercury channels. 1) Arrangement of the recording arms; 2) opening for the second limb of the mercury channel; 3) opening for the vertical bar; 4) hole into which removable bar fits.

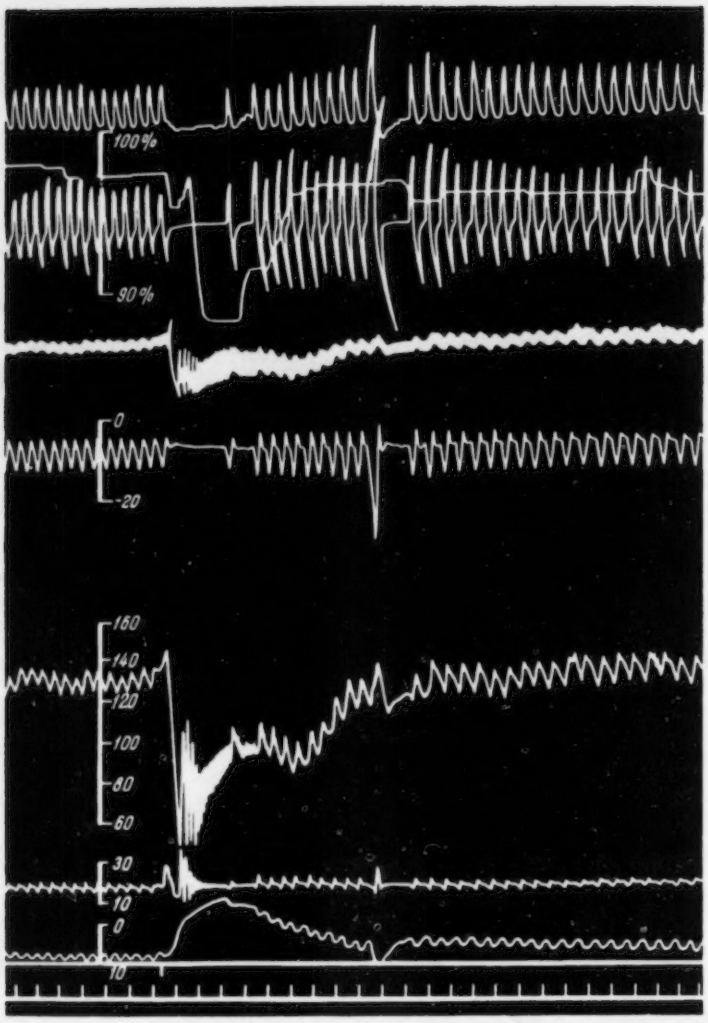

Fig. 3. Kymogram of an experiment on a dog (morphine and oxygen anesthesia). Curves, from top down: respiratory movement of the thorax; blood oxygen saturation in carotid artery; tracheal pressure; pressure in the femoral artery (membrane manometer); intrapleural pressure (in mm water); pressure in the femoral artery (in mm mercury); pressure in the pulmonary artery (in mm mercury, catheter in right external jugular vein); mean central venous pressure (in mm water, catheter in femoral vein); stimulus marker; time marker (5 sec).

The manometer makes it possible to record synchronously and reliably many pressure variations in the circulation, and no account need be taken of calibrating the apparatus at different times, nor of eliminating electrical artifacts.

Many other recording systems may be used simultaneously with the manometer, such as membrane manometers, electromanometers, capsules for recording respiration, or the removable indicator of an oxyhemograph, etc.

An example of a recording is given in Fig. 3.

High frequency electromanometers give a more detailed trace only in the case of the pulse pressure. Naturally, under conditions where a knowledge of the details of the pulse pressure curve is required, an instrument of low inertia and good linearity is required.

SUMMARY

A description is given of a four- and five-channel water-mercury manometer for use in physiology. Glass tubes, styrofoam floats, glass capillaries and other parts are mounted on three metal brackets supported from a vertical rod. Pointers attached to the capillary tubes are guided between stretched parallel threads. The construction ensures accurate alignment to record synchronously the pressures in different parts of the vascular system. The whole width of the kymograph band may be used for each pressure curve. It is usually not necessary to regulate the synchronization of the different channels after changing the kymograph band.

ABBREVIATIONS MOST FREQUENTLY ENCOUNTERED

IN RUSSIAN BIO-SCIENCES LITERATURE

Abbreviation (Transliterated)	Significance
AMN SSSR	Academy of Medical Sciences, USSR
AN SSSR	Academy of Sciences, USSR
BIN	Biological Institute, Botanical Institute
FTI	Institute of Physiotherapy
GONTI	State United Sci.-Tech. Press
GOST	All-Union State Standard
GRRRI	State Roentgenology, Radiology, and Cancer Institute
GTTI	State Technical and Theoretical Literature Press
GU	State University
I Kh°N	Scientific Research Institute of Surgical Neuropathology
IL (IIL)	Foreign Literature Press
IONKh	Inst. Gen. and Inorganic Chemistry (N. S. Kurnakov)
IP	Soil Science Inst. (Acad. Sci. USSR)
ISN (Izd. Sov. Nauk)	Soviet Science Press
Izd.	Press
LEM	Laboratory for Experimental Morphogenesis
LENDVI	Leningrad Inst. of Dermatology and Venereology
LEO	Laboratory of Experimental Zoology
LIKhT	Leningrad Surgical Institute for Tuberculosis and Bone and Joint Diseases
LIPZ	Leningrad Inst. for Study of Occupational Diseases
LIPK	Leningrad Blood Transfusion Institute
Medgiz	State Medical Literature Press
MOPISh	Moscow Society of Apiculture and Sericulture
MVI	Moscow Veterinary Institute
MZdrav	Ministry of Health
MZI	Moscow Zootechnical Institute
LOKhO	Leningrad Society of Orthopedic Surgeons
NIIZ	Scientific Research Institute of Zoology
NINKhI	Scientific Research Institute of Neurosurgery
NIU	Scientific Institute for Fertilizers
NIUIF	Scientific Research Institute of Fertilizers and Insecticides
NIVI	Veterinary Scientific Research Institute
ONTI	United Sci.-Tech. Press
OTI	Division of Technical Information
RBO	Russian Botanical Society
ROP	Russian Society of Pathologists
SANIIRI	Central Asia Scientific Research Institute of Irrigation
SANIISh	Central Asia Scientific Research Institute of Sericulture
TsNII	All-Union Central Scientific Research Institute
TsNTL	Central Scientific and Technical Laboratory
VASKhNIL	All-Union Academy of Agricultural Sciences
VIG	All-Union Institute of Helminthology
VIEM	All-Union Institute of Experimental Medicine
VIR	All-Union Institute of Plant Cultivation
VIUAA	All-Union Institute of Fertilizers, Soil Science, and Agricultural Engineering
VIZR	All-Union Institute of Medical and Pharmaceutical Herbs
VNIRO	All-Union Scientific Institute of Fishing and Oceanography
ZIN	Zoological Inst. (Acad. Sci. USSR)

Note: Abbreviations not on this list and not explained in the translation have been transliterated, no further information about their significance being available to us. - Publisher.

Soviet Journals Available in Cover-to-Cover Translation

ABBREVIATION	RUSSIAN TITLE	TITLE OF TRANSLATION	PUBLISHER	TRANSLATION BEGAN Vol.	Issue	Year
AÉ	Atomnaya énergiya	Soviet Journal of Atomic Energy	Consultants Bureau	1	1	1956
Akust. zh.	Akusticheskii zhurnal	Soviet Physics – Acoustics	American Institute of Physics	1	1	1955
	Antibiotiki	Antibiotics	Consultants Bureau	4	1	1959
Astr(on). zh(urn).	Astronomicheskii zhurnal	Soviet Astronomy–AJ	American Institute of Physics	34	1	1957
Avto(mat). svarka	Avtomaticheskaya svarka	Automatic Welding	British Welding Research Association (London)		1	1959
	Avtomatika i Telemekhanika	Automation and Remote Control	Instrument Society of America	27	1	1956
	Biofizika	Biophysics	National Institutes of Health*		1	1957
	Biokhimiya	Biochemistry	Consultants Bureau	21	1	1956
Byull. éksp(erim). biol. i med.	Byulleten' éksperimental'noi biologii i meditsiny	Bulletin of Experimental Biology and Medicine	Consultants Bureau	41	1	1959
DAN (SSSR) } Dokl(ady) AN SSSR }	Doklady Akademii Nauk SSSR	The translation of this journal is published in sections, as follows:				
Life Sciences		Doklady Biochemistry Section	American Institute of Biological Sciences	106	1	1956
Life Sciences		Doklady Biological Sciences Sections (Includes: Anatomy, biophysics, cytology, ecology, embryology, endocrinology, evolutionary morphology, genetics, histology, hydrobiology microbiology, morphology, parasitology, physiology, zoology sections)	American Institute of Biological Sciences	112	1	1957
Life Sciences		Doklady Botanical Sciences Sections (Includes: Botany, phytopathology, plant anatomy, plant ecology, plant embryology, plant physiology, plant morphology sections)	American Institute of Biological Sciences	112	1	1957
Chemical Sciences		Proceedings of the Academy of Sciences of the USSR, Section: Chemical Technology	Consultants Bureau	106	1	1956
Chemical Sciences		Proceedings of the Academy of Sciences of the USSR, Section: Chemistry	Consultants Bureau	106	1	1956
Chemical Sciences		Proceedings of the Academy of Sciences of the USSR, Section: Physical Chemistry	Consultants Bureau	112	1	1957
Earth Sciences		Doklady Earth Sciences Sections (Includes: Geochemistry, geology, geophysics, hydrogeology, mineralogy, paleontology, petrography, permafrost sections)	American Geological Institute	124	1	1959
Earth Sciences		Proceedings of the Academy of Sciences of the USSR, Section: Geochemistry	Consultants Bureau	106-123	1-6	1957-1958
Earth Sciences		Proceedings of the Academy of Sciences of the USSR, Sections: Geology	Consultants Bureau	106-123	1-6	1957-1958
Mathematics		Doklady Soviet Mathematics	The American Mathematics Society	131	1	1961
Physics		Soviet Physics–Doklady (Includes: Aerodynamics, astronomy, crystallography, cybernetics and control theory, electrical engineering, energetics, fluid mechanics, heat engineering, hydraulics, mathematical physics, mechanics, physics, technical physics, theory of elasticity sections)	American Institute of Physics	106	1	1956
Physics		Proceedings of the Academy of Sciences of the USSR, Applied Physics Sections (does not include mathematical physics or physics sections)	Consultants Bureau	106-117	1	1956-1957
Derevoobrabat. prom-st'.	Derevoobrabatyvayushchaya promyshlennost'	Wood Processing Industry	Timber Development Association (London)		9	1959
	Élektrosvyaz	Telecommunications	Massachusetts Institute of Technology*		1	1957
Éntom(ol). oboz(renie)	Éntomologicheskoe obozrenie	Entomological Review	American Institute of Biological Sciences	38	1	1959
Farmakol. (i) toksikol(ogiya)	Farmakologiya i toksikologiya	Pharmacology and Toxicology	Consultants Bureau	20	1	1957
FMM	Fizika metallov i metallovedenie	Physics of Metals and Metallography	Acta Metallurgica*	5	1	1957
Fiziol. zhurn. SSSR (im. Sechenova)	Fiziologicheskii zhurnal im. I. M. Sechenova	Sechenov Physiological Journal USSR	National Institutes of Health*		1	1957
Fiziol(ogiya) rast.	Fiziologiya rastenii	Plant Physiology	American Institute of Biological Sciences	4	1	1957
	Geokhimiya	Geochemistry	The Geochemical Society		1	1958
FTT	Fizika tverdogo tela	Soviet Physics–Solid State	American Institute of Physics	1	1	1959
Izmerit. tekh(nika)	Izmeritel'naya tekhnika	Measurement Techniques	Instrument Society of America		1	1959
Izv. AN SSSR, O(td). Kh(im). N(auk)	Izvestiya Akademii Nauk SSSR: Otdelenie khimicheskikh nauk	Bulletin of the Academy of Sciences of the USSR: Division of Chemical Sciences	Consultants Bureau		1	1952

continued

Izv. AN SSSR, O(td). T(ekhn). N(auk): Met(all). i top.	(see Met. i top.)				
Izv. AN SSSR Ser. fiz(ich).	Izvestiya Akademii Nauk SSSR: Seriya fizicheskaya	Bulletin of the Academy of Sciences of the USSR: Physical Series	Columbia Technical Translations	1	1954
Izv. AN SSSR Ser. geofiz.	Izvestiya Akademii Nauk SSSR: Seriya geofizicheskaya	Bulletin (Izvestiya) of the Academy of Sciences USSR: Geophysics Series	American Geophysical Union	1	1954
Izv. AN SSSR Ser. geol.	Izvestiya Akademii Nauk SSSR: Seriya geologicheskaya	Izvestiya of the Academy of Sciences of the USSR: Geologic Series	American Geological Institute	1	1958
Kauch. i rez.	Kauchuk i rezina	Soviet Rubber Technology	Research Association of British Rubber Manufacturers	18 1	1959
	Kinetika i kataliz	Kinetics and Catalysis	Consultants Bureau	1 3	1960
	Koks i khimiya	Coke and Chemistry USSR	Coal Tar Research Association (Leeds, England)	1	1958
Kolloidn. zh(urn).	Kolloidnyi zhurnal	Colloid Journal	Consultants Bureau	14 1	1952
	Kristallografiya	Soviet Physics – Crystallography	American Institute of Physics	2 1	1957
Metalov. i term. obrabot. metal.	Metallovedenie i termicheskaya obrabotka metallov	Metal Science and Heat Treatment of Metals	Acta Metallurgica	6 1	1958
	Metallurg	Metallurgist	Acta Metallurgica	1	1957
Met. i top.	Metallurgiya i topliva	Russian Metallurgy and Fuels	Eagle Technical Publications	1	1960
Mikrobiol.	Mikrobiologiya	Microbiology	American Institute of Biological Sciences	26 1	1957
OS	Optika i spektroskopiya	Optics and Spectroscopy	American Institute of Physics	6	1959
	Pochvovedenie	Soviet Soil Science	American Institute of Biological Sciences	1	1958
	Priborostroenie	Instrument Construction	British Scientific Instrument Research Association	1	1959
Pribory i tekhn. éks(perimenta)	Pribory i tekhnika éksperimenta	Instruments and Experimental Techniques	Instrument Society of America	1	1957
Prikl. matem. i mekh.	Prikladnaya matematika i mekhanika	Applied Mathematics and Mechanics	American Society of Mechanical Engineers	1	1958
PTÉ	(see Pribory i tekhn. éks.)				
	Problemy Severa	Problems of the North	National Research Council of Canada		
Radiotekh.	Radiotekhnika	Radio Engineering	Massachusetts Institute of Technology*	12 1	1957
Radiotekh. i élektronika	Radiotekhnika i élektronika	Radio Engineering and Electronics	Massachusetts Institute of Technology*	2 1	1957
	Stanki i instrument	Machines and Tooling	Production Engineering Research Assoc.	1	1959
	Stal'	Stal (in English)	Iron and Steel Institute	1	1959
Stek. i keram.	Steklo i keramika	Glass and Ceramics	Consultants Bureau	13 1	1956
Svaroch. proiz-vo	Svarochnoe proizvodstvo	Welding Production	British Welding Research Association	4	1959
Teor. veroyat. i prim.	Teoriya veroyatnostei i ee primenenie	Theory of Probability and Its Applications	Society for Industrial and Applied Mathematics	1	1956
Tsvet. Metally	Tsvetnye metally	Nonferrous Metals	Primary Sources	1	1960
UFN	Uspekhi fizicheskikh Nauk	Soviet Physics – Uspekhi (partial translation)	American Institute of Physics	66 1	1958
UKh	Uspekhi khimii	Russian Chemical Reviews	The Chemical Society (London)	1	1960
UMN	Uspekhi matematicheskikh nauk	Russian Mathematical Surveys	London Mathematical Society	15 1	1960
Usp. fiz. nauk	(see UFN)				
Usp. khim(ii)	(see UKh)				
Usp. matem. nauk	(see UMN)				
Usp. sovr. biol.	Uspekhi sovremennoi biologii	Russian Review of Biology	Oliver and Boyd	48	1959
Vest. mashinostroeniya	Vestnik mashinostroeniya	Russian Engineering Journal	Production Engineering Research Assoc.	4	1959
Vop. gem. i per. krovi	Voprosy gematologii i perelivaniya krovi	Problems of Hematology and Blood Transfusion	National Institutes of Health*	1	1957
Vop. onk.	Voprosy onkologii	Problems of Oncology	National Institutes of Health*	1	1957
Vop. virusol.	Voprosy virusologii	Problems of Virology	National Institutes of Health*	1	1957
Zav(odsk). lab(oratoriya)	Zavodskaya laboratoriya	Industrial Laboratory	Instrument Society of America	25 1	1959
ZhAKh Zh. anal(it). khimii	Zhurnal analiticheskoi khimii	Journal of Analytical Chemistry USSR	Consultants Bureau	7 1	1952
ZhETF Zh. éksperim. i teor. fiz.	Zhurnal éksperimental'noi i theoreticheskoi fiziki	Soviet Physics–JETP	American Institute of Physics	28 1	1955
ZhFKh Zh. fiz. khimii	Zhurnal fizicheskoi khimii	Russian Journal of Physical Chemistry	The Chemical Society (London)	7	1959
ZhMÉI Zh(urn). mikrobiol. épidemiol. i immunobiol.	Zhurnal mikrobiologii, épidemiologii i immunobiologii	Journal of Microbiology, Epidemiology and Immunobiology	National Institutes of Health*	1	1957
ZhNKh Zh(urn). neorgan(ich). khim(ii)	Zhurnal neorganicheskoi khimii	The Russian Journal of Inorganic Chemistry	The Chemical Society (London)	1	1959
ZhOKh Zh(urn). obshch(ei) khimii	Zhurnal obshchei khimii	Journal of General Chemistry USSR	Consultants Bureau	19 1	1949
ZhPKh Zh(urn). prikl. khimii	Zhurnal prikladnoi khimii	Journal of Applied Chemistry USSR	Consultants Bureau	23 1	1950
ZhSKh Zh(urn). strukt. khimii	Zhurnal strukturnoi khimii	Journal of Structural Chemistry	Consultants Bureau	1 1	1960
ZhTF Zh(urn). tekhn. fiz.	Zhurnal teknicheskoi fiziki	Soviet Physics–Technical Physics	American Institute of Physics	26 1	1956
Zh(urn). vyssh. nervn. deyat. (im. Pavlova)	Zhurnal vysshei nervnoi deyatel'nosti (im. I. P. Pavlova)	Pavlov Journal of Higher Nervous Activity	National Institutes of Health*	1	1958

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  Program for the Day
  The Environment and Its Importance
  Physical Hypnogenic Factors
  The Use of Hypnotic Drugs
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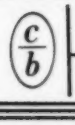

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